

GROWTH AND IMBIBITION.

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(*Read April 13, 1917.*)

GENERAL CONSIDERATIONS.

The chief purpose of the studies described in the present paper has been to correlate some of the more striking features of growth in plants with the action of contributory factors, and to resolve this complex process into its constituent reactions so far as might be possible.

New viewpoints have been sought by the reduction and analyses of continuous series of measurements of the entire course of enlargement of single organs or members. Experimental species were chosen concerning which much was known as to their respiration, transpiration, imbibition capacity and chemical composition. The daily, seasonal and developmental variations in such matters as carbohydrate content, acidity and swelling capacity of some of the plants had already been the subject of various investigations at the Desert Laboratory, and additional determinations were made in the course of the work. The final or actual increase which is measurable as growth, by weight or dimensions is predominantly a hydration or imbibition process as the increment to any growing cell or embryonic region is at least 99 per cent. water. There is immediate necessity therefore for a study of factors influencing imbibition. Whatever theory of colloidal structure may be adopted, there is no reason for supposing that the interpolation or absorption of water in a complex mixture of such substances is different in the plant cell from what it might be in similar material in the laboratory. The protoplast and its envelopes are undoubtedly a complicated mixture of colloids in a state of more or less constant change.

A successful search was instituted for mixtures which would show the same general imbibition phenomena as the living plant.

Gelatine alone has been found to furnish valuable analogies in the study of the action of animal tissues. It is not adequate for the vegetable protoplast however. Mixtures consisting largely of the amorphous condensed carbohydrates such as agar to which is added a small proportion of albumen or amino-acid are found to respond to the action of acids, alkalies and salts in a manner similar to that of the plant.

Some new conceptions of the general nature of respiration and its correlation with growth have been made possible. The origin and fate of the sugars, particularly the pentosans, have been made the object of extended experimentation, and the results obtained are not the least important of those presented herewith. Most of the attempts which have been made to ascertain the essential nature of growth have been made on the assumption that it is a single, simple or unified process. Thus for example, much attention has been concentrated upon fixing the lower and upper limits of growth with regard to temperature, and recently much has been written concerning the temperature coefficient. A number of authors concur in the assertion that within a certain range, generally between 15° C. and 30° C., the rate of acceleration is one which follows the van't Hof law of doubling or tripling for every rise of 10° C., it being agreed that no such conformity is shown in the extreme upper and lower ranges of temperature. This partial or accidental agreement of smoothed curves of growth with those depicting the course of simple reactions has diverted attention more than once from the fundamental fact that growth depends primarily on respiration, imbibition and osmosis. Respiration is essentially a complicated swirl of sugar disintegration processes which may be influenced in any one of its parts by the oxidation potential, by the dearth of material or over-accumulation of products in any part of the complex. The concentration of the various reaction products may exert their own direct effect on imbibition and consequent enlargement. In addition to, and partly dependent upon the imbibition phenomena, elongation may be modified by such factors as water-loss. Thus for instance, growth upon a rising temperature may reach a point where, as a result of temperature, the water-loss would temporarily be greater than the supply, with the result that a cessation, slacken-

ing or shortening would ensue until an adequate supply reached the expanding region.

The proposal of Rahn¹ to explain the relation of growth to temperature upon the basis of a direct integration of enzymatic action and enzyme destruction does not seem adequate. It is true that among the reactions upon which the growth of plants depends are syntheses or renewals of thermo-labile material, and upon metabolism possibly including oxidation of carbohydrates. Each of the separate processes or reactions, enzymatic or otherwise, goes on at a rate determined by the temperature, and by the concentration of its products, and to an extent limited by the amount of material brought into its reactions. The extent to which, for example, the sugars are oxidized determines the degree of acidity or alkalinity of the cell thus affecting its water relations in a very serious manner. Also as will be shown later, the swelling of colloids, and presumably the growth capacity of a cell, may be modified by proteins, while its volume or measurable variation in volume is at all times a function of the balance between water-accession and water-loss.

The cell itself may be considered as a mass of colloidal material variously altered from the globular by pressure and contacts. The outermost layer being of greater density or compactness is usually designated as a membrane, and much has been written during the past few years concerning the permeability and the modifiable semi-permeability of such structures. The meristematic or embryonic cell with the action of which we are chiefly concerned in growth, is in its earlier stages dense and shows none of the cavities or clear spaces which form such a large part of the volume of a mature cell, while the relatively large nucleus shows even greater density.

The enlargement of this mass consists to an extent as great as 98 or 99 per cent. in swelling by the imbibition of water. The rate, extent and total amount of such swelling will be determined by the character of the colloidal mixture, by salts, acidity or alkalinity of the solutions present, and only to a very slight extent by osmosis as this process takes place in colloids. Hence turgidity may play a very minor part in the earlier stages.

¹ Rahn, O., "Der Einfluss der Temperatur und der Gifte auf Enzymwirkung, Gärung, und Wachstum," *Biochem. Ztschrift.*, 27: 351, 1916.

With increase in size vacuoles begin to appear. The active cell is usually conceived as a sac with irregular strands of cytoplasm extending from the peripheral layers of protoplasm, the nucleus being variously placed in this irregular mass. The vacuoles or spaces appearing colorless in living cells and clear in preparations are taken to be sacs containing electrolytes or other dissolved material. The capacity of these dissolved substances to absorb water osmotically tends to increase their volume and cause distension resulting in turgidity or swelling of the cell and in rigidity of the organ when whole tracts or layers act in this manner. Turgidity has hitherto been held to account for the entire expansion of growth as noted above. It is now apparent, however, that we are in a position to draw a slightly different picture of the mechanical features of the cell in what may be termed the second stage. In addition to the denser colloids of the wall, the lining layer of protoplasm, and the nuclear structures, it is known that even in the clear regions of the cell there are emulsions and that the entire cell is a mass of gels of different composition and varying degrees of dispersion. The cell may take water into the vacuoles by the osmotic action of the electrolytes, but the entire mass tends to swell as would a mixture of protein, cellulose, agar, gum arabic, starch and other substances, and such masses may be modified by transpiration or direct loss of water.

The first recognition of the differential action of acidity and alkalinity appears to have been expressed by Spoehr and Estill who say:²

It has become evident that the total swelling of plants like *Opuntia blakeana* and *O. discata* in dilute solutions of acids, alkalis, and salts represents the summation of independent reactions of various material to these reagents. Thus, solutions of acids, alkalies and salts influence the swelling and growth of these plants by affecting: (1) the hydration of the protoplasts; (2) the material that goes to make up the cell-wall and fibro-vascular system; (3) the permeability and osmotic properties of the plasma-membrane. It has been found that these three factors can act independently and even in opposite directions. Great differences were found in these respects in different portions of the same cactus joint and between young and mature ones; the colloidal material of the former showed much greater swelling than the latter in all solutions, and the excess of swelling in acid media above that in

² Report Dept. Bot. Res. Carnegie Inst. of Wash. for 1915, p. 66:

alkaline media or distilled water was much greater in the young joints. Of interest is the observation that the colloidal material from mature joints which have been freed as much as possible from the fibro-vascular strands showed a diminution in volume in weak alkaline solution.

Mr. E. R. Long also working at the Desert Laboratory made some tests of this matter and found that the swelling capacity of sections of *Opuntia discata* as determined by weighing, was less in acidified than in neutral solutions and that the swelling was sometimes less in alkaline solutions than in distilled water.³ These results suggested that it would not longer be profitable to consider the plant as a protein gel and that some comprehensive tests would be necessary to establish the general colloidal character of growing parts.

This mistake had been made by Borowikow⁴ who assumed that plant cells would grow in an acid condition like a mass of gelatine, showing the greatest imbibition of water in acids.

The action of plant tissues having been determined, it was attempted to make up mixtures of colloids similar to those occurring in the plant which might show parallel reactions. The technique and results of measurements of the swelling of plant tissues and of plates of colloidal mixtures will be given in a separate section of this paper. It may be said in this place that some highly profitable comparisons are made possible by the data obtained.

The effort to compound colloidal mixtures which might simulate living material was extended to include additions of other proteins beside gelatine, such as egg-albumin, bean-albumin and of amino-acids, together with complex condensed carbohydrates as agar. This was rewarded by results which show that small proportions of soluble proteins or albumens added to gelatine-agar mixtures decrease the water-absorbing capacity of these physical analogues of the protoplasts in the presence of electrolytes, and suggest the highly interesting possibility that the growth-enlargement of the cell might be definitely checked or terminated by the passage of such albuminous emulsions from the nucleus to the cytoplasm. The

³ "Growth and Colloid Hydration in Cacti," *Botan. Gazette*, 59: 491, 1914.

⁴ *Biochem. Ztschrift.*, 48: 230-246 and 50: 119-128, 1913.

actual quantities necessary to produce the action described in a later section of this paper would be small and in some cases lie beyond detection by ordinary microchemical or cytological methods.

Some of the earlier results obtained by a study of the growth of opuntias have already been described by the senior author.⁵

The comparison of the action of *Opuntia* with that of roots and stems of peas, beans, wheat, corn and oats, etc., led to the inference that many of the accepted conclusions concerning growth rested upon data obtained from material representing a specialized or narrow range of physiological action. An inspection of the records of measurement shows that no distinction is usually made as to whether the elongation is due to the action of one embryonic tract as in the case of roots or hypocotyls, or of many as in the case of stems and leaves. It is also to be noted that even in the simplified action of roots the elongation is a different expression from that of such an organ as a sporangiophore. Measurements of growth of the tip of a root include the imbibitional swelling of younger cells, the combined swelling and turgidity effects of older protoplasts, with all of the modifications due to salinity, acidity, alkalinity, character of the respiration, permeability of the membranes and albumen condition.

The elongation of a stem may include the total action of several internodes representing various stages of the grand period of growth, while it may be assumed that in some cases the records of leaves represent the variations in length of these organs and of one or more internodes.

The experimental material used in the investigation described in the present paper included the conventional subjects, *Zea* and *Triticum*, which were tested for purposes of orientation. Chief attention however was given to succulents which have long been known to present a type of respiration different from that of the leafy and slender-bodied plants. Furthermore, the massive bodies of the succulents presented characteristic body-temperature conditions which could be readily measured.

The flattened shoots of *Opuntia* present a single growing region

⁵ See MacDougal, "Mechanism and Conditions of Growth," *Mem. N. Y. Bot. Garden*, 6: 5-26, 1916.

of great volume which is active through a long period. Such plants are amenable to chemical analyses, and have mechanical qualities which make it possible to place the apex in bearing upon an auxograph lever and secure a continuous record of its activity during the entire period of enlargement, as well as of the subsequent variations in length. Detailed studies of the course of transpiration and respiration of these plants have been made at the Desert Laboratory, and the available information on these subjects was of great usefulness in interpreting growth and other changes in volume. A *cylindropuntia* was also tested in order to ascertain possible differences due to mechanical form. Both kinds have a type of respiration in which a notable accumulation of acids occur at temperatures in the lower part of the tonic range and in darkness. The leaves of *Mesembryanthemum* presented different morphological features, but a similar type of respiration. The massive globose and cylindrical stems of *Echinocactus* and *Carnegiea* were also used as their metabolism is of a character which does not result in any notable accumulation of residual acids in any part of the respiratory mesh. The meristem region in both is entirely terminal, and some detailed studies of the fate of the carbohydrates and of the non-auxetic variations in thickness and length as well as of transpiration had been previously made.

GROWTH OF OPUNTIA.

These preliminary studies brought out the fact that the flattened joints of the opuntias undergo most rapid growth during the daylight period, coincident with decreasing acidity and lessened transpiration, and that actual shrinkage occurs in maturing joints as the result of reactions which are masked during the period of most active growth. The entire development of about forty flattened joints has been followed from bud to maturity, and the changes in volume of members in an adult condition have been noted for long periods under varying conditions. The swelling of hundreds of specimens from growing and mature joints were measured, and an extended series of records of the action of gelatine, agar, albumen and cactus mucilage in acids, alkalies and salt solutions made.

Unless otherwise stated, all of the growth records included in

the present paper were made by an improved form of the auxograph described by the senior author in 1916. The changes in the instrument were for the purpose of securing greater delicacy and accuracy. Twelve of these instruments as described on page 330 of the present paper were available.

The joints of *Opuntia* occupy the better part of a month in developing from a length of 15 mm. with a volume of a few cu. cm. to a length of 200 mm. with a volume of perhaps 150–200 cu. cm. The entire mass of this member remains in an embryonic or elongating condition until nearly mature, the development of woody or permanent tissue being very light during the first 20–25 days. It may be conceived therefore as a thick plate of protoplasts in all stages of development from the earliest when enlargement is a result of imbibition alone, to a state approaching maturity where the osmotic action of the electrolytes in the vacuoles maintains a turgidity indicated by the fact that expressed juice shows a possible pressure of 5 to 8 atmospheres. Temperatures were established or taken by thermometers with thin bulbs thrust into similar members in close proximity, and as has been mentioned elsewhere in this paper, the temperatures cited are those of the plant instead of the air as is the case in many of the papers dealing with growth (Fig. 1).

A feature prominently emphasized by our studies is the interdependence of effects. The influence of any one environic agency is of course affected by the intensity of action of other agencies influencing the plant. This is well illustrated by the behavior of *O. discata* No. 14, with respect to temperature. A young joint in the form of a flattened naked bud of this plant was followed from Feb. 28, 1916, to maturity, about April 30, 1916, and then its further alterations in volume until June 7, 1916, at which time disks were taken and the swelling capacity of the tissues determined. Measurements of growth for every moment of 62 days, of reversible alterations 38 days and of final hydration capacity are available together with body and air-temperatures.

The plant stood on a cement bench near the glass of the southern end of a greenhouse and exposed to normal illumination as modified by the glass. It was kept in bearing with a precision auxograph in such manner as to reduce errors to a minimum. The following

entries are cited from the notes accompanying the auxographic tracings:

Elongating at 12° C. and below on March 2, with the air at about

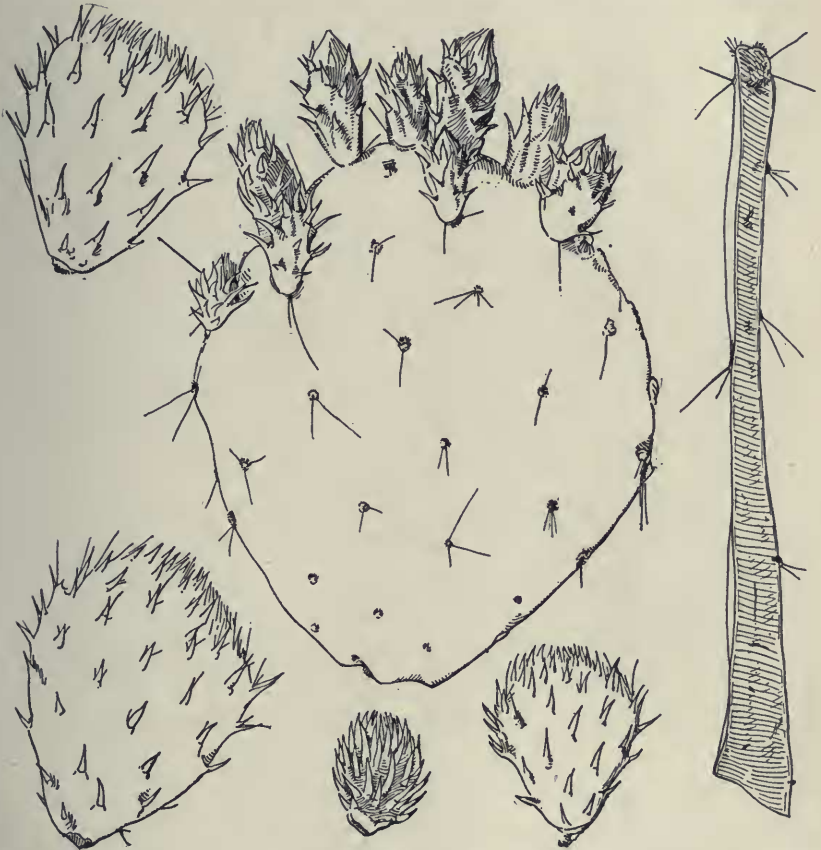


FIG. 1. Joints of *Opuntia Sp.* The youngest stage at which growth measurements were begun is illustrated by the small figure at the bottom. Successive stages are denoted by size. The largest figure is that of a mature joint bearing flower buds. Longitudinal section of joint on the right. Growth throughout the entire joint during its development is denoted by the increasing distances between the nodes denoted by the clusters of spines. About one third actual size.

the same temperature: Elongation began on March 23, after a night of shortening, at a temperature of 18° C., and under similar conditions, but with air temperature falling to 9° C. growth began

at 14° or 15° C. on the 24th. Growth began at 14° C. on March 25 and at some point between 15.5° C. and 17.5° C. on the 26th. Growth began at 17° C. after a night of shortening, at 19° C. on the 31st after a night of shortening, at 15° C. on April 1, at 18° C. on the 2d and 3d, at 14° C. on the 4th and 5th, at 18° C. on the 6th, 16° C. on the 7th, at 18° C. on the 9th, 19° C. on the 11th, 18° C. on the 12th, at 13° C. on the 13th, at 17° C. on the 14th, 22° C. on the 18th, 17° C. on the 19th, above 20° C. on the 19th, and 21° C. on the 20th.

Similar experiences with many other growing joints are in our records. Thus we have the entry that on March 31 all growing joints under observation began elongation at temperatures ranging from 15° to 19° C. This single growing member began elongation in temperatures rising from 9° to 10° C. early in its development to 12° to 22° C. in its more advanced stages. Another joint, No. 2, began its daily growth at temperatures as follows :

March 24, 10:00 A.M.	21.5° C.
28, 8:30	"	22° C.
29, 8:40	"	23° C.
31, 9:40	"	19° C.
April 1, 8:40	"	20° C.
2, 9:00	"	21.5° C.
3, 8:40	"	20° C.
4, 8:30	"	21.5° C.
5, 8:40	"	22.5° C.
6, 8:35	"	23° C.
7, 8:30	"	23° C.
9, 8:40	"	24.5° C.
10, 8:30	"	25° C.
11, 8:50	"	17° C.
12, 8:00	"	19° C.
13, 11:30	"	16° C.
14, 10:30	"	16.5° C.

The temperatures of the body at which growth ceased likewise showed great variation as illustrated by the behavior of No. 14. Thus on March 28, 1916, elongation ceased abruptly when it reached 40° C., and the temperature of the air was 26° C. Growth stopped at 35° C. at 1:30 P.M. on the 25th; at 28° C. at 2:30 P.M. on March 30, the temperature having been above that point since 10 A.M.; at

39° C. at 1 P.M. March 29; at 35° C. at 1:30 P.M. April 4; at 32° C. at 3 P.M. April 5; at 36° C. at 1:30 P.M. April 6; 37° C. at 2 P.M. April 7. The upper temperature limit is given in other records included in the present paper, the extreme highest recorded being 51.5° C.

A second series of cultures for observation of growth and temperature were arranged at the Coastal Laboratory, Carmel, California, in the summer of 1916. Preparations consisting of an old joint with roots were placed in a dark chamber in which temperature could be controlled. The basal joints from which the buds arose held a supply of reserve material quite adequate for the development of the etiolated shoots. Some of the latter were growing vigorously six months after the close of the tests described.

These tests were made under conditions different from those encountered by the plants in the open in two important essentials, viz.: the temperature did not rise to a daily maximum and fall to a nightly minimum, but was maintained at fixed levels or varied as described and the action of light was excluded except for brief intervals when observations were being made. The effect of such conditions would be to exclude the disintegrating action of light on the acids resulting from respiration, and also to make photosynthesis impossible. Both of these features contribute to the daily variation in growth of plants in the open. Growth of shoots in darkness may be taken to be normal otherwise, so far as respiration and imbibition are concerned.

An etiolated shoot of *Opuntia discata* which had arisen in the dark chamber in which it had been placed in May, 1916, having a length of 65 mm. and a width of 15 mm., was chosen for the first test, which was duplicated by later ones. The container in which the plant stood was fastened firmly in place and an auxograph was brought into contact with it adjusted to record alterations in length magnified twenty times. A small thermometer with thin bulb of the "clinical" type was inserted in the old joint near the base of the young shoot and its readings taken to be those of the growing organ. The difference between the two could be only very slight. The amount of growth displayed by the shoot on five successive days was 1.2, 1, .1, 1 and 1.1 mm. at temperatures of 17°–18° C., July

21-25, 1916. Current was now turned on an electric heater, July 25, 10 A.M., and the *Opuntia* reached 25° C. about 6 P.M.

July 27, 1916: 4:30 P.M.—A growth of nearly 3 mm. had occurred in the previous 24 hours at a temperature of 24° C. and 25° C.

28, 11:00 A.M. Growth during the previous 18.5 hours was at rate of 3.6 mm. per day, 25° C. 3:00 P.M. Growth for previous 4 hours was at rate of 3.9 mm. per day, 25° C.

Current off and plant cooled to 18° C. at midnight—in 9 hours.

29, 8:00 A.M. Growth during previous 8 hours was at rate of 3.3 mm. per day at 18° C. 10:00 A.M. Growth of .2 mm. in 2 hours was at rate of 2.4 mm. per day, 18-17° C., which was double the rate displayed at the same temperature before being heated. 4:00 P.M. Growth at rate of 2 mm. daily during previous 6 hours at 19° C.

30, 7:00 A.M. Growth during previous ten hours was at rate of 2.4 mm. daily at 19° C.

31, 7:00 A.M. Growth of 2.4 mm. during previous 24 hours at 18° C.

Aug. 1, 6:30 A.M. Growth in previous 19 hours was at rate of 2.6 mm. daily, at 18-19° C.

The plant failing to return to the initial rate of about 1 mm. daily, the heater was again put in action and the plant had a temperature of 28° C. at 11 A.M. Growth during this rise of 9° C. in 4.5 hours was 1 mm. or at rate of about 5.4 mm. daily.

The temperature was held constant to within a degree but the rate was 6 mm. daily during the first 6 hours, then 7.2 mm. per day during the next 3.5 hours.

2, 8:00 A.M. Growth at rate of 8.04 mm. per day during previous 11.5 hours at 28° C. 2:00 P.M. Rate during previous 5 hours 10.8 mm. daily at 27-28° C. 4:00 P.M. Rate during previous 2 hours 12 mm. daily at 28° C. 9:00 P.M. Rate 9 mm. daily during previous 5 hours.

Heat was now cut off and the temperature fell to 16° C. in 4 hours.

3, 8:00 A.M. Rate of 2.9 mm. daily during previous 7 hours at

16° C 2:00 P.M. Rate 2.4 mm. daily during previous 6 hours at 18–16° C.

Heat was again turned on, and the control set at 25° C. This point was reached in 2 hours. The rate during this time was 4.8 mm. daily. 9:00 P.M. Rate 2.7 mm. daily during previous 3 hours at 25° C.

4, 8:00 A.M. Rate 6.6 mm. daily during previous 9 hours at 25.5° C. 11:00 A.M. Rate 5.6 mm. daily during previous 3 hours at 25° C.

Control reset and temperatures of 32° C. were reached by 3 P.M., the rate during this period of 4 hours being 6 mm. daily. The temperature rose from 32° C. to 36° C. during the next 3 hours.

5, 12 Noon. Rate 9.6 mm. daily during previous 4.5 hours at 34–35° C. 3:15 P.M. Rate 9.9 mm. during previous 3.25 hours.

Current was now cut to reduce temperature as follows: 3:45 P.M. Temperature 26° C. 5:30 P.M. Rate .5 mm. in 1.25 hours at 29° C. at rate of 9.5 mm. daily. 8:00 P.M. Rate 8.1 mm. daily during previous 2.5 hours at 27° C.

6, 8:00 A.M. Rate 7.2 mm. per day during previous 12 hours at 24° C.

Earthquake disarranged record. Current cut off. 7:15 P.M. Temperature 19° C.

7, 8:00 A.M. Rate of 3.2 mm. per day in previous 12 hours at 18° C., which fell to 2.8 mm. per day during following 2 hours at 18° C.

The shoot was now 12.2 cm. in length. Record was discontinued until August 14, during which time the plant stood at 16–18° C. and gained 18 mm. in length, or about 3 mm. per day.

17. Current on heater at 2 P.M. resulted in a temperature of 23° C. at 9:15 P.M.

18, 8:00 A.M. Rate of 2.1 mm. during previous 10 hours at 23° C.

19, 8:00 A.M. Rate of 3.3 mm. daily during previous 15.5 hours at 25° C.

23, 8:00 A.M. Plant had stood at 25° C. for 4 days. Rate during previous 16 hours was 5.7 mm. per day at 25° C. 12 Noon. Rate 7.8 mm. per day during previous 4 hours at 25° C.

Watered and record disturbed for 2 hours. 5:00 P.M. Rate 7.8 mm. per day at 25° C.

24, 8:00 A.M. Rate 5.7 mm. per day for previous 15 hours at 25° C.

25, 8:00 A.M. Rate 6 mm. daily, 25° C.

Control reset and as temperature of the body rose the rate calculated in 2 hour intervals increased from 8.4 mm. at 27° C. to 9.6 mm. at 29° C. and 10.8 mm. at 29.5° C.

26, 10:00 A.M. Rate was substantially maintained at 29° C., being 9.6 mm. for the forenoon. 2:00 P.M. Rate 11.4 mm. daily, 31.5° C. 4:00 P.M. Rate 11.4 mm. daily, 32° C.

27, 8:00 A.M. Rate 3.9 mm. daily at 17° C. 11:00 A.M. Rate 5.8 mm. daily at 18° C. 10:00 P.M. Rate 3.8 mm. daily at 18° C.

28, 8:00 A.M. Current on for higher temperature. 9:15 A.M. Temperature of 32° C. was reached and 39° C. at 11:30 A.M. One hour later at 12:30 midday, the rate was 7.2 mm. per day at 39° C. 1:30 P.M. Rate 4.8 mm. per day at 40° C. 2:30 P.M. Rate 3.6 mm. per day at 40° C. 3:30 P.M. Rate 4.8 mm. per day at 40° C. 5:30 P.M. Rate 1.8 mm. per day at 41.5° C. 7:30 P.M. No growth had taken place in the previous 2 hours. 9:30 P.M. Rate of 3.6 mm. daily, the temperature having fallen to 36° C.

Another shoot of the plant used in making the preceding record being available, an auxograph was put in bearing with it when a length of about 150 mm. had been reached on August 29, 1916. The rate varied from about 15.6 mm. to 20.4 mm. daily at 35° C. to 37° C. The temperature was raised from 36° C. to 47° C. in an hour and a half on the third day, elongation stopping when this point was reached. During the second hour and a half the temperature was allowed to fall to 43° C., growth being resumed above 43° C. and continued at a rate varying from 10.8 mm. daily in the first hour, 6.6 mm. daily during the following four hours, to 8.4 mm. daily during the sixth hour. The temperature being raised to 46° C. in twenty minutes, growth stopped at that point. Shortening took place during the following hour and a half at temperatures of 46° C. to 48.5° C., but ceased as the temperature was brought back to 44° C. at some point above that temperature. The shoot ap-

peared to be slightly limp, suggesting that elongation and shortening might be a matter of the balance between water accession and loss.

The shoot was now subjected to a temperature above 43° C. continuously for two days, the maximum being 52° C. Its body

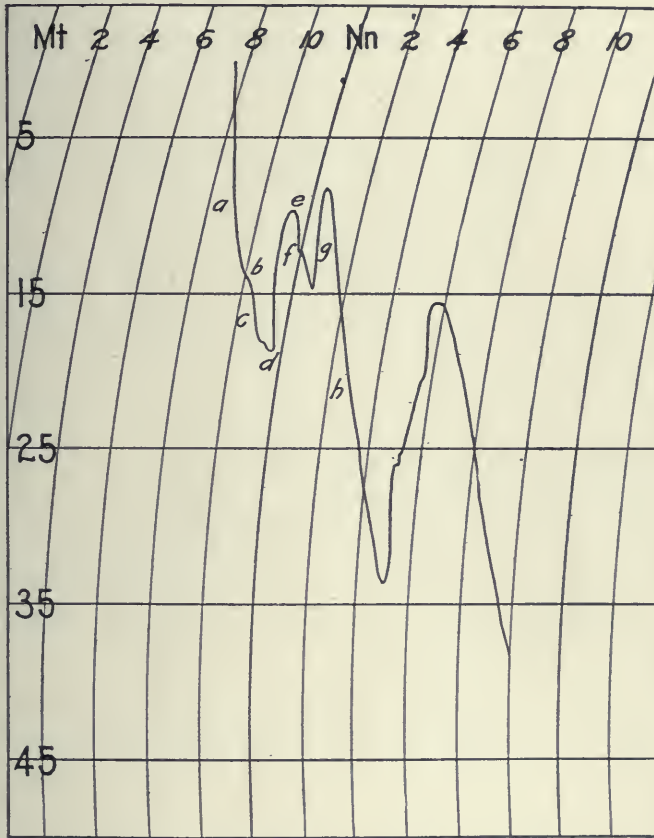


FIG. 2. Auxographic tracing of variations in length of shoots of *Opuntia* at high temperatures in dark room at Carmel, September 1, 1916. The sheet is ruled into two-hour periods by arcs and the 10 mm. horizontal lines of the millimeter sheets are reproduced. The variation in length is magnified 26 times. (a) Downward movement of pen 7:30 A.M. to 9:40 A.M. denoting growth at temperatures of the stem of 45° to 49° C. (b) Growth checked for 20 minutes at 49° C. (c) Growth resumed at temperature of 49° C. (d) Shortening at 48.5° to 52° C. (e) Stationary at 50.5° C. (f) Growing at temperatures of 48° to 49° C. (g) Shortening at 49° C. (h) Growing at 38° to 41° C. (i) Shortening at 49° C.

temperature was then brought down to 25° C. and after twelve hours at this point it showed rates of 5.4 mm., 6.6 mm., 6.6 mm. and 5.4 mm. daily for two days as measured at two hour intervals. The temperature was now raised to 35° C. at which the rate was 16.4 mm. to 16.8 mm. daily for an entire day. This rate was fairly duplicated on a second day, but with a somewhat wider variation, the rate ranging from 15.6 mm. daily to 22.2 mm. daily (Fig. 2).

The etiolated shoot of a second *Opuntia* elongated as follows:

Rate of 1	mm. daily at	19° C.
Rate of 1.3	mm. daily at	19° C.
Rate of .7	mm. daily at	15° C.
Rate of .93	mm. daily at	15-18° C.
Rate of 1.08	mm. daily at	17-18° C.
Rate of 1.44	mm. daily at	18-19.5° C.

Rates of 1, 2, 1.2, 1.15, .85 and 1 mm. per day at 16-18° C.

The preparation was moved into control chamber and the following results were obtained:

Rates of 2.9, 3, 3, and 3 mm. daily at 26° C.

Rates of 3.2 and 3.44 mm. daily at 26° C.

Rate of 4.2 mm. daily at 29° C.

Rates of 3.6, 6.7 and 9.6 mm. daily at 30° C.

Rates of 11.4, 11.4, 8.4, 9.2, and 11.4 mm. daily at 31.5-32° C.

The heat was now cut off and rates of 4 mm. daily at 17° C. were displayed.

Rate of 5.7 mm. daily at 18.5° C.

Rate of 5.3 mm. daily at 19° C.

The temperature being raised again from 18° C. at 8 A.M. to 39° C. at 12:30 midday, a rate of 8.4 mm. daily was displayed during the first hour.

The continuation of similar temperatures during two days was attended by sustained rate of 18.6 to 19.2 mm. daily (37-38° C.). After three days at this temperature it was raised from 37° C. to 45° C. in 1.5 hours during which time the elongation was at the rate of 13.2 mm. daily, and growth stopped entirely at 45° C. Some shortening now ensued, but at the end of an hour and a half elongation began again at 46° C., and was maintained at the rate of 25 mm. per day for an hour, and the total for the four succeeding hours at 46° C. was a rate of about 20 mm. daily, which was not exceeded by any rate at lower temperatures. The rate during the sixth hour

rose to 20.4 mm. daily at 45° C. The temperature being raised to 49° C. during the next hour, elongation ensued at the rate of 18.7 mm. daily until it was checked at 48.5° C. The period of checking was not measured accurately, but after an hour with the temperature still between 48° C. to 46° C. the rate was 19.2 mm. daily. It is thus to be seen that the maximum is maintained up to very near the point of actual cessation of growth, an experience duplicated scores of times with green plants in the glass house at Tucson. It was noted that the air temperature was 40° C. and 41° C. when the plant was at its maximum of 48° C. and 49° C. Similar differences have probably gone unnoted in the observations made by many workers.

After the experiences described above the plant remained at 45° C. and 46° C. over night without calibration. Measurements begun at 7:30 A.M. at 45° C. Elongation during the hour and a half in which the temperature rose a degree and a half (to 47.5° C.) amounted to .95 mm. at a rate of about 15 mm. daily. Continued rise of temperature was accompanied by lessened growth which did not cease altogether until 49° C. was reached. Elongation was resumed at this temperature however after 20 minutes, but was checked again. The temperature was now raised to 52° C. for a half hour with an air temperature of 43° C. Reducing the temperature to 49° C. with an air temperature of 41° C. resulted in a growth of .4 mm. at a rate of 9.6 mm. daily. Similar changes resulted in starting and checking growth in much the same manner. At the end of the day the chamber was allowed to cool to give the plant a constant temperature of 25° C. and after standing at this temperature for 12 hours measurements were made to determine the rate at this point.

The rate at 25° C. on the following day varied from 9.6 mm. daily in the morning to 12.6 mm. at 26.5° C., then to 8.8 mm. daily at the close of the day at 26° C. No measurements were made at night but during the two hours beginning at 8 A.M. the rate was 13.2 mm. daily at 25° C., after which the temperature was raised to get values for the next ten degree interval. A rate of 17.9 mm. daily was found between 5 P.M. and 10:30 P.M. at temperatures of 34-37° C.

The plant now stood over night at 34-37° C., at a rate of 27 mm.

daily, which decreased to 21.6 mm. daily at a temperature kept within a narrow range at 35° C. This now being gradually raised to 40° C. in a six hour interval the rate at first fell to 19.2 mm. daily, then rose to 29 mm. daily, the maximum when measured at two hour intervals. Almost any rise in temperature up to about 46° C. seemed to be followed by a temporary acceleration in rate.

Two younger shoots had arisen from the second *Opuntia* during these tests and had attained a length of about 30 mm. during the previous ten days. These were designated as "A" and "B."

The temperature of these young shoots was between 25-45° C. during most of this time, and for a few hours rose to 52° C. as described in connection with the tests with other shoots. Separate auxographs were put in bearing with the two shoots and thermometers were arranged to take the temperature of the basal joint from which they arose and of one of the other growing shoots near by.

The interest attached to the detail of the growth of these two shoots warrants the transcription of the complete record.

Sept. 5, 1916, continued: As soon as the instruments were adjusted, the temperatures which were standing at 30° C. were raised by the use of an additional heater giving the following records:

	A	B	
8:00 A.M.	Both growing.		30° C.
9:00 "	Both growing.		32
10:00 "	Both growing.		37-40
10:30 "	Both growing.		41-42
11:00 "	Both growing.		43
12:30 P.M.	Both growing.		46-47
	Growth stopped in both.		
12:45 "	Growth starting.		43
1:30 "	Both growing.		46.5
2:00 "	Both growing.		47
2:15 "	Both growing.		50
4:00 "	Stopped.		
4:35 "	A little growth in both.		46.5
5:15 "	Some growth.		49 +
6:30 "	Some growth.		49 +
7:30 "	Some growth.		49 +
9:30 "	Stationary.		48 +
6, 7:30 A.M.	Shortening but had grown until four hours before.		51° C.

	8:00 A.M.	Stationary.		48
	8:30 "	Stationary.		45-46
	9:00 "	Just beginning to grow.		46.5-47
	10:00 "	Shortening.		46-48
	11:00 "	Stationary.		45-46.5
	12:00 M.	Stationary.		47
	1:00 P.M.	Stationary.		48
	1:30 "	Stationary.		47.5
	5:00 "	Shortening.		49-50
	6:00 "	Shortening.		48.5-49
	7:30 "	Shortening.		49
7,	7:30 A.M.	Plants had shorteneded until pen was above sheet and the temperature now stood at 51° C.		
	8:00 "			45-47 C.
	8:45 "	No action.		43-44
	9:00 "	No action.		42-43
	9:20 "	No action.		41.5-42.5
	9:50 "	No action.		42
	10:30 "	No action.		42
	11:00 "	No action.		40.5-41.5
	12:30 P.M.	No action.		42-43
	2:00 "	No action.		39
	2:30 "	No action.		38
	3:00 "	No action.		37.5
	3:50 "	Growth beginning.		37
	5:00 "	Growth checked.		38
8,	7:30 A.M.	5.8 mm. daily.	6.8 mm. daily.	31
	10:00 "	3.8 mm. daily.	3.3 mm. daily.	31.5
	10:30 "	Shortening.		32
	4:00 P.M.	2.4 mm. daily.	5.2 mm. daily.	32
	9:00 "	3.0 mm. daily.	3.6 mm. daily.	31
9,	7:30 A.M.	4.3 mm. daily.	5.0 mm. daily.	30
	10:00 "	1.9 mm. daily.	5.3 mm. daily.	30
		Reset.		
	1:30 P.M.	1.9 mm. daily.	2.9 mm. daily.	30
		2.4 mm. daily.	4.8 mm. daily.	31
	9:30 "	1.9 mm. daily.	4.0 mm. daily.	31
10,	8:00 A.M.	1.7 mm. daily.	4.8 mm. daily.	29
		(For 10.5 hours.)	(For 10.5 hours.)	
		Temperature raised to 39° at noon.		
	12:15 P.M.	Checking.	6.0 mm. daily.	39
	5:00 "	Stopped.	8.0 mm. daily.	39
	9:15 "	Stopped.	7.9 mm. daily.	39
11,	7:30 A.M.	Stopped.	8.4 mm.	39-37
		(During entire night.)		
	10:00 "	Stopped.	7.2 mm. daily.	36
	11:00 "	Stopped.	12.0 mm. daily.	37

	2:00 P.M.	Stopped.	10.8 mm. daily.	41
	3:30 "	Stopped.	8.0 mm. daily.	38
	7:30 "	Stopped.	8.1 mm. daily.	37
12,	8:00 A.M.	Stopped.	8.6 mm.	37
			(All night.)	
	11:00 "	Stopped.	13.6 mm. daily.	36.5
	1:30 P.M.	Stopped.	12.3 mm. daily.	37
	3:30 "	Stopped.	9.0 mm. daily.	39
	5:00 "	Stopped.	12.8 mm. daily.	38
	6:00 "	Stopped.	10.8 mm. daily.	38
	7:30 "	Stopped.	13.6 mm. daily.	38
	9:30 "	Stopped.	15.0 mm. daily.	38

The behavior of green opuntias in daylight was tested in March, 1917, at Tucson. Preparations consisting of a rooted joint from which a flower bud was arising were placed in the south end of a glass house in an equatorial position. The temperature of the body rose to 40° C. and 43° C. by the heat of the sun after 1 P.M. Additional heat was supplied by tungsten incandescent lights so that the temperature was raised to 49° C. in an hour at which point elongation ceased. The temperature following same rising curve reached 51.5° C. a half hour later at which elongation was resumed, and was maintained at temperatures of 51° C. to 51.5° C. for an hour and a half when it ceased. This behavior is in accordance with that of etiolated shoots illustrated in Fig. 2. On the following day the temperature near midday, which was above 40° C. by the sun's heat, was raised to 48° C. and 49° C. for a half hour by additional heat from a tungsten incandescent light bulb. Growth continued at a rate near the maximum. In an additional preparation a bulb for heating not regulated properly raised the temperature of a portion of the joint 75° C. for a few minutes, resulting in the death of a sector within the next two days. The young shoot arising from the margin of the injured area probably reached a temperature of 65° C. or 70° C. as some of the outer leaves were blackened. Growth was checked at once but was resumed eighteen hours later and continued for two days with the customary mid-afternoon shortening.

The gas interchange and variation in the concentration of the residual acids has been worked out in detail in *Opuntia versicolor*. Some available data show that the platyopuntia used so extensively

in this work present an identical type of respiration. That the course of growth was similar in its general features was evidenced by the records of the two plants which were under observation for some time. Elongation begins with full daylight and assumes its highest rate near midday and then checks abruptly about 1 P.M. Shrinkage continues from this time until daylight of the following morning. The end of the growing period is marked by a decrease of the midday elongation and by increased shrinkage which equal-

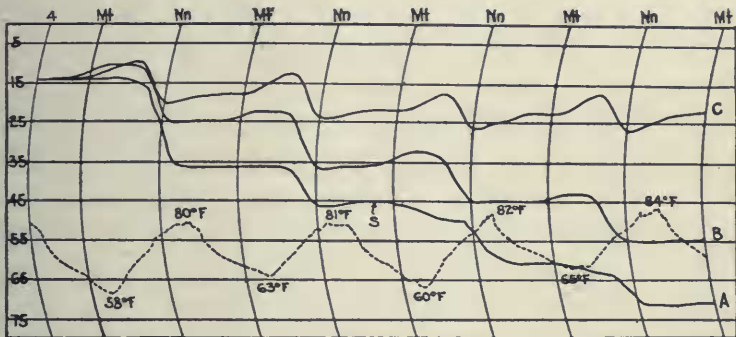


FIG. 3. Auxographic records of growth of joint of *Opuntia versicolor*.

A. Record April 10 to April 15, 1916, rapid midday elongation of joint near the maximum of its grand period. The first occurrence of shortening at S.

B. Record from April 18 to April 22, 1916. Slight diminution of daily growth and accentuated contraction at night. The temperature record applies to this period.

C. Record from April 25 to April 29, 1916. Increasing reversible variation in length with cessation of growth.

ize each other while allowing a great total variation in length (see Fig. 3).

The general facts as to alterations in volume of *Opuntia* by growth and other changes, including shrinkage, are in accordance with those previously described.⁶ Elongation takes place chiefly in the first half of the day both in mature and growing joints. Shrinkage, slackening or stoppage of growth ensues after midday and continues for a varying period which may extend until the following morning. The type of respiration of these plants is one in

⁶ MacDougal, D. T., "Mechanism and Conditions of Growth," *Mem. N. Y. Bot. Garden*, 6: 5-26, 1916.

which residual acids accumulate at low temperatures and in darkness. Acidosis decreases imbibition. Growth beginning with sunrise shows an acceleration parallel to the disintegration of the clogging acids and the rising capacity for imbibition, till midday only. The retardation after this may not be ascribed to lessened power of imbibition or to increased transpiration as water-loss is not greater during this time and the capacity of the plant continues to increase until near the end of the daylight period. The cause of the retardation cannot be identified with the direct action of light, nor does it seem warranted to assume that the "supply of building material" becomes exhausted, as was previously suggested by the senior author. The nature of the stoppage suggests the inhibiting action of respiratory products or the destruction of an enzyme. Respiration in *Opuntia* is profoundly affected by light as has been shown by its effect on acid-accumulation and destruction. Yet no immediate effects were secured by exposure of growing members to the action of mercury vapor quartz lamps with an intensity equivalent to normal sunlight at 2 meters distance, for periods of one to three hours. It is noteworthy that the characteristic retardation or stoppage does not take place in the first few days of the development of the bud, and that the leaves of *Mesembryanthemum* exhibit a similar behavior. The young shoots of *Opuntia* in this stage are not more than 8 to 12 mm. in length, 1 to 2 mm. in thickness and are all but hidden by the slender conical leaves. The joint as well as the leaves are in a state of extreme imbibition. The character of the respiration under such conditions is in all probability such that acids do not accumulate and other by-products are modified with the result that the daily decrease in imbibition capacity is not experienced. A similar behavior attends the development of the flower buds. That retardation and stoppage as observed in hundreds of instances could not be ascribed primarily to temperature seemed to be established by the great variation in the point at which growth might begin or cease.

Growth began on rising temperatures at 9° C. to 25° C. in the same green plants on different days at Tucson and was noted at 50° C. in flower buds. The continued rise of the temperature resulted in a stoppage of elongation at temperatures between 26° C.

and 43° C., in the plant which has been cited, with a final limit of temperatures of the body of 51.5° C. in some other extreme cases.

Growth of etiolated shoots of a nearly related species in a dark room was 1 mm. or less per day in members at body temperatures of 15° C. or 16° C. Rates of 2 to 2.6 mm. daily at 16° C. to 18° C. were followed by 8 to 12 mm. daily at 27° C. and 28° C. yielding values of 3 to 4 mm. for a rise of 10° C. Rates of 5.6 to 7.8 mm. daily at 24° C. and 25° C. being compared with 8.4 to 10.2 mm. daily at 29° C. to 32° C. show a similar coefficient at 29° C. to 31.5° C. The meager records at 35° C. and 36° C. yield rates of 10.2 to 13.2 mm. daily. Observed rates at temperatures above 32° C. or 33° C. in the shoot showing such rates were not readily to be integrated with these results, and growth ceased at 41.5° C. in the shoot yielding them.

The second shoot of the same plant showed rates of .85 to 1.2 mm. daily at 16° C. to 18° C.; 2.9 to 3.4 mm. at 26° C., and 13.2 mm. daily at 35° C.; 20.4 mm. daily at 46° C., and 18.5 mm. daily at 48.5° C.

The highest observed rates, both in green plants and in etiolated shoots, were those immediately preceding cessation of growth; a daily occurrence in plants exposed to normal sunlight.

Accepted conclusions as to growth include an *optimum* at which growth proceeds continuously at a high rate, and above which the rate is higher for a brief period then falls off. Some of the records are conformable to such ideas and others are not. The two shoots of the same plant subjected to the same treatment did not agree in this matter, as may be seen in the preceding pages. It is conceded that our experiments were not arranged to bear critically on this point. It is to be noted that growing shoots in the open may cease to elongate at temperatures as low as 26° C. which would be below any *optimum* hitherto suggested. Hundreds of observations of such cessations under external conditions supposedly favorable to continuous growth are available. The facts in question seem to lessen the importance and the usefulness of the term *optimum temperature*.

The results of measurements of growth of the apical part of the

globular *Echinocactus* and of the cylindrical *Carnegiea* afford some interesting comparisons, since both are massive succulents, but present a type of respiration something different from that of *Opuntia*.⁷

The spines of *Echinocactus* arise from special meristem tracts lateral to the growing point, and as the growth is wholly basal the rigid tips afford an excellent bearing for an auxograph arm. A preparation was kept under observation at a point some distance from the walls of a greenhouse late in April, 1916. Temperatures of the body near the surface were taken by a thermometer with a thin bulb left in place during the course of the observation. Growth began at 22° C. to 24° C., about 8 A.M., continuing during the warm daylight period and until nearly 8 P.M. Nothing higher than 37° C. was shown by the body. The daily rate varied from zero to .05 mm. per hour and no retractions were discernible. The length remained fairly constant when growth ceased. The temperature of the body of this plant did not fall below about 14° C. during any part of the period.

The same plant was available for experimental purposes in

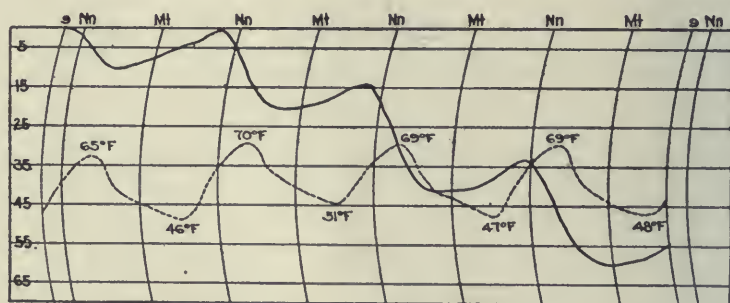


FIG. 4. Auxographic record of variations in length of spine of *Echinocactus*, March 13 to March 17, 1917. Shortening from 8 P.M. to 8 A.M. due to low temperature. $\times 10$.

March, 1916. The cluster of spines, the tips of which had emerged for a length of 4 to 6 mm. in 1916, began to show freshly colored sections at their bases indicative of elongation and one of these was brought into bearing in the cup-shaped end of the vertical arm of

⁷ MacDougal, "The End-results of Desiccation and Starvation of Succulent Plants," *Physiological Researches*, Vol. 1, No. 7, 1915.

an auxograph. The preparation was placed near the south end of an unheated glass house with the result that the temperature of the body fell as low as 4° C. at 7 A.M., and reached a point at which growth ceased at about 8 P.M. The steadily decreasing temperature was accompanied by a shrinkage—due in all probability to lessened imbibition capacity as a result of low temperature. Resumption of growth took place in the forenoon at temperatures about identical with those of the previous year. The total daily growth amounted to as much as 1.25 mm. to 1.5 mm. daily all of which was made between 9 A.M. and 8 P.M. (Fig. 4).

The record of growth of *Carnegiea* included measurements of the variations in the length of the spine as well as of coincident readings of the swelling of the apical region of the stem near the base of the spine.

Elongation of the spine on daily rising temperatures began at temperatures of 24° C., 18° C., 18° C., 15° C., 13° C. and 13° C. on sepa-

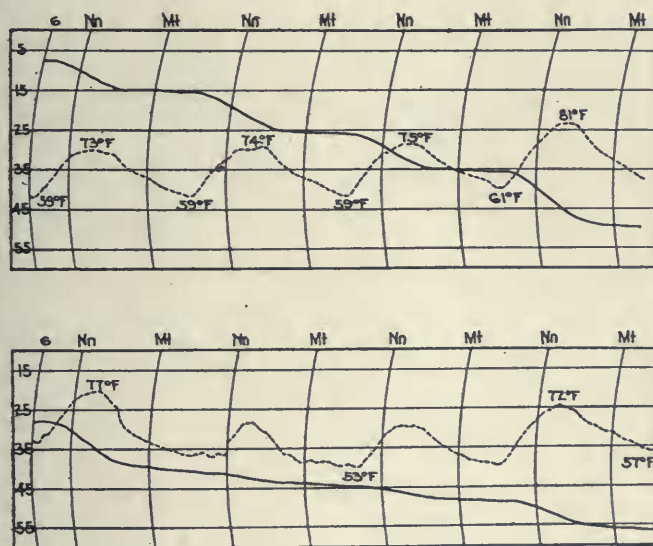


FIG. 5. Auxographic record of elongation of spine of *Carnegiea* April 3 to April 10, 1916, showing nocturnal cessation of growth. Dotted line shows maxima, minima and course of air temperature (upper half of cut). Record of growth of spine of *Carnegiea*, April 12 to April 16, 1916. Continuous growth with only slight variation in rate. Dotted line shows maxima, minima and course of air temperature (lower half of cut).

rate days and was very active at 32° C. A period of continuous elongation of the spine was comprised between April 10 to 17, 1916, during which time the air temperature ranged between 14° C. and 28° C. The temperature of the body coincided with the lower night temperature of the air and did not rise above 32° C. (Fig. 5).

The maximum enlargement of the spine was at rate of .075 mm. per hour, while that of the neighboring apical tract was not more than a third of this rate. After the spine had reached nearly mature length the apical tissue accelerated showing a rate as .088 mm. per hour. Growth began on rising temperatures of 15° C. and above and was observed at 40° C. of the body. The main part of the growth took place in the daytime and no action directly attributable to light effects could be detected.

Echinocactus and *Carnegiea* are active during the period in which the temperature is within the tonic range, as taken from thermometers inserted in the tissues. This implies that such plants grow during the daylight period in the open and as far into the night as the temperature permits, the maximum rate being attained during midday. Numerous tests show but little variation in the acidity of *Echinocactus* and *Carnegiea*, and it is to be inferred that the respiration of the sugars is of a kind in which the disintegration is carried through to its final limits.

A number of records of growth of the succulent leaves of *Mesembryanthemum inequilaterale* were obtained for comparison with *Opuntia*, *Carnegiea* and *Echinocactus*. Determinations of the acidity of the sap show that while the total range is not as great as that found in *Opuntia versicolor* by Richards,⁸ yet the daily course of variation is marked, as may be seen from the following measurements of *Mesembryanthemum*.

ACIDITY IN CUBIC CENTIMETERS OF N/100 NaOH.

	Pure Juice per C.cm.	December 7.		Pure Juice per C.cm.	December 8.	
		Total Acidity per Gm. Dry Material.	Total Acidity per Gm. Fresh Material.		Total Acidity per Gm. Dry Material.	Total Acidity per Gm. Fresh Material.
8:00 A.M....	.0280	1.584	.0356	.0273	1.072	.029
12:00 M.....	.0279	1.509	.0351	.0225	1.091	.0241
4:30 P.M....	.0232	1.191	.0264	.0205	1.056	.0275

⁸ "Acidity and Gas Interchange in Cacti," Publ. No. 209, Carnegie Inst. of Washington, 1915.

The leaves are triangular in cross section and as the pairs emerge from the sheathing bases of the antecedent pair the inner or upper faces are appressed. The upright position implied is held until a half or a third of their length is attained. The tips of a pair were harnessed together and being turgid and firm were arranged to press upward on the bearing lever of the auxograph.

The general features of the daily behavior of this plant were quite similar to those of *Opuntia* in that elongation accelerated in mid-forenoon, about 9 to 11 A.M., and continued until 1 to 3 P.M., when it was checked and a shrinkage ensued which generally ended at 5 or 6 P.M. or sunset. After this time temperature being favorable a low rate of growth continued through the night and until the daily acceleration occurred a few hours after sunrise.

The daily course of transpiration has not been determined, but it is allowable to assume that the imbibition capacity of the growing regions is lessened by acidity as it is in *Opuntia*.

GROWTH OF WHEAT (*Triticum*) AND CORN (*Zea*).

A great amount of data obtained by the measurement of the elongation of *Triticum* is available. The figures have been obtained chiefly by the measurement of numbers of organs for a brief period. The so-called critical temperature points have been obtained by taking averages of the performance of several plants. The facts of importance in connection with the present paper are those which have been obtained by analyses of the march of growth from day to day. Similar methods were used with corn (*Zea*).

Varieties of these two plants cultivated in the region of the Desert Laboratory were selected, and grains were germinated in an unheated glass house. The temperatures given were obtained by shaded mercurial thermometers and are Fahrenheit scale.

The bases of the plantlets were fixed in place by layers of plaster poured on the surface of the soil. The tips of leaves which had emerged to a length of 10 to 15 mm. were brought into the field of a horizontal microscope and the variation in length measured at half hour intervals so far as it was possible to do so. The leaves were maintained in a vertical position by a requisite number of horizontal glass rods with a minimum of shading effect.

The increments measured are of course inclusive of the elongation of the base of the leaf and of the internode from which it arises, as well as of any residual action of internodes below, consequently a figure illustrative of the grand period of growth of a single member could not be plotted from the data given. (Measurements showing a beginning of decreasing rate are given in bold-faced type.)

VARIATIONS IN LENGTH OF LEAF OF "ALTAR CORN" (*Zea*).

1914

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.	
April 8	11:30 A.M.	0	85° F.	0 mm.	
	12:30 P.M.	2.6	87	2.6	
	1:00	3.7	90	2.2	
	1:30	4.9	90	2.4	
	2:00	6.1	91	2.4	
	2:30	7.2	91	2.2	
	3:00	8.1	92	1.8	
	3:30	9.0	90	1.8	
	4:00	9.8	90	1.6	
	4:30	10.5	89	1.4	
	5:30	10.7	85	1.2	
	April 9 (18 hours)	11:30 A.M.	43.3	86.5	1.8
12:00 Noon		44.9	88	3.2	
12:30 P.M.		46.5	88	3.2	
1:00		48.1	90	3.2	
1:30		49.3	90	2.4	
2:00		50.8	89	3.0	
2:30		52.3	87	3.0	
3:00		53.4	86	2.2	
3:30		54.7	85	2.6	
4:00		55.7	83	2.0	
5:00		57.2	83	1.5	
5:30		58.1	82	1.8	
6:00		59.4	80	2.6	
6:30		60.6	78	2.4	
		Reset at	2.8		0.0
7:00			4.0	77	2.4
7:30		4.9	76	1.8	
8:00		5.9	75	2.0	
April 10 (13 hours)	9:00 A.M.		74	1.9	
	9:30	32.8	76	2.4	
	10:00	34.2	79	2.8	

VARIATIONS IN LENGTH OF LEAF OF "ALTAR CORN" (*Zea*).—*Continued*.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.
	10:30	35.6	80	2.8
	11:00	37.1	82	3.0
	11:30	38.6	83	3.0
	12:00 Noon	40.3	85	3.4
	12:30 P.M.	41.8	87	3.0
	1:00	43.4	87	3.2
	1:30	44.8	88	2.8
	2:00	46.2	89	2.8
	2:30	47.5	89	2.6
	3:00	48.8	88	2.6
	3:30	50.1	88	2.6
	4:30	52.5	87	2.4
	5:00	53.4	85	1.8
	5:30	54.9	84	3.0
April 11	11:30 A.M.			
	Reset at	0.2	84	—
	12:00 Noon	2.3	84	4.2
	12:30 P.M.	4.3	86	4.0
	1:00	6.3	86	4.0
	1:30	7.9	86	3.2
	2:00	9.6	87.5	3.4
	4:00	16.4	88	3.2
	4:30	17.7	87	2.6
	5:00	18.9	85	2.4
April 12 (15 hours)	8:00 A.M.	53.2	63	2.3
	Reset at	0.2		
	9:00	1.8	74	1.6
	10:30	6.0	83	3.4
	11:00	8.0	85	4.0
	2:30 P.M.	18.5	90	3.5
	3:30	21.0	90	3.5
	5:30	25.3	91.5	2.2
	8:00	32.0	80.0	2.7
April 13 (13.5 hours)	9:30 A.M.	56.0	78.0	1.8
	Reset at	0.8	78	—
	10:00	1.7	81	1.8
	10:30	2.8	84	2.2
	11:00	3.7	86	1.8
	11:30 (watered)	4.9	88	2.4
	12:00 Noon	7.3	90	4.8
	12:30 P.M.	10.8	92	7.0
	1:00	13.5	92	5.4
	1:30	16.2	93	5.4

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VARIATIONS IN LENGTH OF LEAF OF "ALTAR CORN" (*Zea*).—Continued.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.
	2:00	18.4	94	4.4
	2:30	20.4	95	4.0
	3:00	22.2	95	3.6
	3:30	23.7	94	3.0
	4:00	25.5	94.5	3.6
	4:30	26.9	93.5	2.8
	5:00	28.2	92	2.6
	5:30	29.7	91	3.0
April 14 (16 hours)	9:30 A.M.	74.0	81	2.8
	Reset at	0.0	81	—
	10:00	3.7	83	3.4
	10:30	5.3	85.5	3.2
	11:00	7.4	88	4.2
	11:30	9.6	90	4.4
	12:00 Noon	11.5	91	3.8
	12:30 P.M.	13.6	93	4.2
	1:00	15.5	95.5	3.8
	1:30	17.3	97	3.6
	2:00	19.1	98	3.6
	2:30		98	3.0
	3:00	21.9	98.5	2.6
	3:30	23.6	97	3.4
	4:00	24.7	97	2.2
April 15 (17.5 hours)	9:30 A.M.	63.0	83	2.2
	Reset at	1.4	83	—
	10:00	3.2	86	3.6
	10:30	4.9	89.5	3.4
	11:00	6.7	92	3.6
	11:30	8.5	94	3.6
	12:00 Noon	10.4	95.5	3.8
	12:30 P.M.	12.0	97	3.2
	1:00	13.3	98	2.6
	1:30	14.4	99	2.2
	2:00	15.6	99.5	2.4
	3:00	17.8	99	2.2
	3:30	18.7	97	1.8
	Reset	0.6	97	—
April 16 (19 hours)	10:30 A.M.	37.8	86	1.5
	11:00	39.2	88	2.8
	11:30	40.4	89	2.4
	12:00 Noon	41.4	90	2.0
	12:30 P.M.	42.5	91.5	2.2

VARIATIONS IN LENGTH OF LEAF OF "ALTAR CORN" (*Zea*).—Continued.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.
	1:00	43.5	93	2.0
	1:30	44.4	93	1.8
	2:00	45.1	93	1.4
	3:00	46.8	93	1.7
	4:00	48.1	92	1.3
	5:00	49.1	90	1.0
	6:00	50.7	89	1.6
	Reset	15.4	76	—
April 17				
	9:00 A.M.	30.4	72	1.0
	10:00	31.4	76	1.0
	11:00	32.4	79.5	1.0
	(watered)			
	1:00 P.M.	34.6	84	1.1
	2:15	36.0	85	1.1
	3:15	38.8	85	2.0
	4:30	37.6	82	—
	5:30	38.4	80	.8
	Reset	23.1	—	—
April 18 (16 hours)				
	9:30 A.M.	30.3	78	.45
	10:30	30.9	81	.6
	11:30	31.6	84.5	.7
	12:30 P.M.	32.5	89	.9
	1:30	32.9	91.5	.4
	2:30	33.4	92	.4
	3:30	34.0	91	.5
	4:30	34.4	90	.4
	5:30	34.9	85	.5
April 19 (16.5 hours)				
	10:00 A.M.	41.9	83	.4
	11:00	42.3	88	.4
	12:00 Noon	42.8	90	.4
	3:30 P.M.	43.4	95	.2
	4:45	43.7	95	.2
April 20				
	9:30 A.M.	48.0	81.5	.25
	11:00	48.0	90	.00
	2:00 P.M.	48.0	98	.00
	5:30	48.0	93	.00
April 21 (16 hours)				
	9:30 A.M.	52.6	77	.3 —
	2:00 P.M.	54.1	78	.3 +
April 22 (19.25 hours)				
	11:15 A.M.	58.8	74	.24
April 23				
	1:00 P.M.	69.2	83	.4

GROWTH OF "TURKEY RED" WHEAT (*Triticum*), MARCH, 1914.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.	
				— mm.	
March 19	11:00 A.M.	0.0	70° F.	—	
	11:30	.8	70	1.6	
	12:00 Noon	1.3	66	1.0	
	12:30 P.M.	1.5	65	.4	
	1:00	1.8	64	.6	
	1:30	2.1	63	.6	
	2:30	2.4	63	.3	
	3:00	2.5	62	.2	
	3:30	2.6	62	.2	
	4:30	2.6	62	.0	
	5:30	2.6	60	.0	
		Reset at	0.0	—	—
	March 20 (5.5 hours)	9:00 A.M.	9.1	54	1.6
		9:30	9.6	56.5	1.0
10:00		10.6	59	2.0	
10:30		11.4	61	1.6	
11:00		12.1	63	1.4	
11:30		12.8	64	1.4	
12:00 Noon		13.6	65	1.6	
12:30 P.M.		14.5	65	1.8	
1:00		15.4	65.5	1.8	
1:30		16.1	69.5	1.4	
2:00		17.2	70	2.2	
2:30		18.2	69	2.0	
3:00		18.9	70	1.4	
3:30		20.0	70	2.2	
4:00		21.1	70.5	2.2	
4:30		22.1	69.5	2.0	
5:00		23.0	68.5	1.8	
March 21 (13.5 hours)	5:30	23.9	68.5	1.8	
	9:00 A.M.	42.7	62	1.2	
		(Total length of leaf-blade 49 mm.)			
	9:30	43.3	65	1.2	
	10:00	44.1	68	1.6	
	10:30	45.4	71	2.6	
	11:00	46.6	73.5	2.4	
	11:30	48.3	76.5	3.4	
	12:00 Noon	49.6	78	2.6	
	12:30 P.M.	51.0	80	2.8	
	1:00	52.4	82	2.8	
	1:30	53.7	82	2.6	
	2:00	54.6	81.5	1.8	
	2:30	55.7	82	2.2	
3:00	56.9	82.5	2.4		
3:30	58.2	82	2.6		

GROWTH OF "TURKEY RED" WHEAT (*Triticum*).—Continued.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.
	4:00	59.3	82	2.2
	4:30	60.4	80	2.2
	5:00	70.2	78	1.6
	Reset at	0.0	—	—
March 22 (16.25 hours)	9:15 A.M. (Total length 91.5 mm.)	23.2	68	1.4
	9:45	24.5	70	2.6
	10:15	26.2	72	3.4
	10:45	27.5	75	2.6
	11:15	28.6	76.5	2.2
	11:45	29.7	79	2.2
	12:15 P.M.	31.2	81	3.0
	12:45	32.8	82	3.2
	12:45 Reset	35.4	82	—
	1:15	36.8	84	2.8
	1:45	38.4	84.5	3.2
	2:15	39.9	84	3.0
	2:45	42.2	84	4.6
	3:15	43.5	84	2.6
	3:45	45.1	84	3.2
	4:15	46.2	82	2.2
	4:45	47.2	82	2.0
	5:15	48.3	81	2.2
March 23 (16.25 hours)	9:30 A.M. (Total length 147 mm.)	70.0	72	1.3
	Reset	0.0	72	—
	10:00	2.2	74	3.2
	10:30	3.9	76	3.4
	11:00	5.2	78	2.6
	11:30	6.3	81	2.2
	12:00 Noon	7.8	82	3.0
	12:30 P.M.	9.3	83.5	3.0
	1:00	10.6	86	2.6
	1:30	12.0	86.5	2.8
	2:00	13.5	87	3.0
	2:30	14.6	83.5	2.2
	3:00	15.8	84	2.4
	3:30	17.2	84	2.8
	4:00	18.6	81.5	2.8
March 24 (17 hours)	9:30 A.M. (Total length 194.5 mm.)	7.0	70	—
	10:00	7.8	72	1.6
	10:30	9.4	74	3.2
	11:00	10.6	75	2.4
	11:30	12.2	81	3.2

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GROWTH OF "TURKEY RED" WHEAT (*Triticum*).—Continued.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.
	12:00 Noon	13.5	82	2.6
	12:30 P.M.	15.0	80	3.0
	1:00	16.4	81.5	2.8
	1:30	18.0	83	3.2
	2:00	19.2	84	2.4
	2:30	20.8	83	3.2
	3:00	22.2	83	2.8
	3:30	23.4	83	2.4
	4:00	24.7	82	2.6
	4:30	26.0	82	2.6
	8:00	34.4	77	2.4
March 25	9:30 A.M.	0.3	72.5	—
	(Total length 244.5 mm.)			
	10:00	1.4	74	2.2
	10:30	2.9	76	3.0
	11:00	4.4	77	2.8
	11:30	5.6	78	2.4
	12:00 Noon	7.3	81	3.4
	12:30 P.M.	8.6	83	2.6
	1:00	10.0	85	2.8
	1:30	11.3	85	2.6
	2:00	12.6	85	2.6
	3:00	16.6	86	4.0
	4:00	17.9	85	1.3
	4:30	19.4	84	3.0
	5:00	20.7	83	2.6
	5:30	21.9	81	2.4
March 26	9:30 A.M.	40.4	74	1.1
(16 hours)	(Total length 295.5 mm.)			
	10:00	41.5	75	2.2
	10:30	42.7	72	2.4
	11:00	44.0	73	2.6
	11:30	45.4	75	2.8
	12:00 Noon	46.7	75	2.6
	12:30 P.M.	48.8	76	2.2
	1:00	49.1	77	.6
	1:30	50.7	78.5	3.2
	2:00	51.8	80	2.2
	2:30	53.3	82	3.0
	3:00	54.4	82	2.2
	3:30	55.7	82	2.6
	4:00	57.1	80	2.8
	4:30	58.1	80	2.0
March 27	9:00 A.M.	0.7	64	
(16 hours)	(Total length 334.5 mm.)			

GROWTH OF "TURKEY RED" WHEAT (*Triticum*).—Continued.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.
	9:30	1.6	64	1.8
	10:00	2.2	67	1.2
	3:30 P.M.	13.2	88.5	2.0
	4:00	14.2	87	2.0
	4:30	15.2	81	2.0
	5:00	16.0	80	1.6
	5:30	16.9	79	1.8
March 28 (13.5 hours)	9:00 A.M.	35.2	67	1.2
	(Total length 369 mm.)			
	9:30	36.1	69	1.8
	10:00	37.2	68.5	2.2
	10:30	38.2	68.5	2.0
	11:00	39.1	72	1.8
	11:30	40.3	74	2.4
	12:00 Noon	41.6	74	2.6
	12:30 P.M.	42.7	74	2.2
	1:30	44.7	70	4.0
	2:00	46.0	71	2.6
	2:30	47.1	69	2.2
	3:30	48.7	65	1.6
	5:00	50.9	65	1.5
	5:30	51.6	64.5	.7
	6:00	52.3	63	.7
	6:30	52.9	61	.6
	7:00	53.5	60	.6
	7:30	53.9	59	.4
	9:00	55.5	56	1.0
March 29 (9.5 hours)	6:30 A.M.	0.4	53	—
	(Total length 400 mm.)			
	7:00	0.7	53	.3
	7:30	1.0	53	.3
	8:00	1.8	54	.8
	8:30	2.5	55	.7
	9:00	3.2	56	.7
	9:30	3.9	59	.7
	10:00	4.7	62	.8
	3:00 P.M.	14.4	66	1.9
	3:30	15.2	68	.8
	4:00	16.1	70.5	.9
	4:30	17.1	70	1.0
	5:00	17.7	69.5	.6
	5:30	18.6	69.5	—
March 30 (16 hours)	9:30 A.M.	37.9	65	1.2
	10:00	38.9	65	2.0
	10:30	39.5	64	1.2

GROWTH OF "TURKEY RED" WHEAT (*Triticum*).—Concluded.

Date.	Hour.	Scale Reading.	Air Temperature.	Rate Per Hour.
	11:00	40.2	67.5	1.4
	11:30	41.5	71	2.6
	12:00 Noon	42.4	71	1.8
	12:30 P.M.	43.5	72.5	2.2
	1:00	44.4	72	1.8
	1:30	45.5	72.5	2.2
	2:00	47.4	76	1.8
	2:30	47.4	75	2.0
	3:00	48.3	73	1.8
	3:30	48.9	75	1.2
	4:00	49.9	76	2.0
	4:30	50.8	76	1.8
	5:00	51.6	75	1.6
	5:30	52.4	75	1.6
March 31 (16.25 hours)	9:45 A.M.	12.6	70	—
	(Total length 467 mm.)			
	10:15	13.0	72	.8
	10:45	13.5	74	1.0
	11:15	13.9	75.5	.8
	11:45	14.3	77	.8
	12:15 P.M.	14.6	80	.6
	1:00	15.3	81.5	.9
	2:00	15.7	84	.4
	4:00	16.3	83	.3
	6:00	16.5	75	.1
April 1	9:30 A.M. (15.5 hours—growth .1 mm.; stopped). Total length 470 mm.			

Retardation of growth of *Zea* and *Triticum* occurs at more than one place in the temperature scale and at different times of the day, as may be seen from the inspection of the bold-faced figures on the preceding pages. An uneven rate of elongation was particularly noticeable in *Triticum*, although displayed by *Zea* as well. It was thought that the irregularity might be due to a sagging of the leaf blade which would cause its tip to move with a varying rate across the field. Similar leaves attached to the bearing arm of an auxograph under a stretching tension traced an undulating line indicative of similar irregularities (Fig. 6). Cessation of growth, especially in some of the instances in *Zea*, may be reasonably attributed to a direct temperature effect, especially in the cases in which the thermometer stood at 30° C. to 35° C. for extended periods. In the

greater number of instances particularly in *Triticum*, no such explanation could be deemed adequate, and the matter is referred to varying imbibition capacity coincident with alternations of acidity, alkalinity and neutralization (see page 309).

The highest rate that was maintained for some time by *Zea* was found to lie between 27° C. and 30° C. The elongation of the leaf

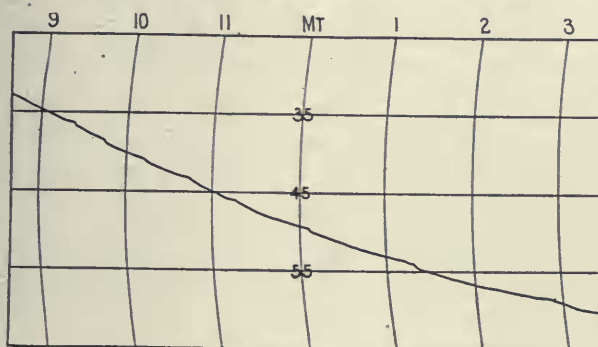


FIG. 6. Auxographic record of growth of leaf of wheat (*Triticum*) for six hours showing sudden alterations in rate of elongation. The pen moves downward with elongation. Actual variation in length. $\times 15$.

of *Triticum* was erratic and retardations were numerous and occurring at all temperatures between 15° C. and 30° C. It is not possible to fix upon any limits of temperature within which growth might be continuous in this plant. It is obvious that "secondary" maxima might readily be derived from data of this character.

No retardations occurred except after 11 A.M. in either *Zea* or *Triticum* and while *Zea* showed an acceleration late in the day after retardation at high temperatures, *Triticum* did not. The tonic range of the two plants is of course not identical. Wheat grows at a lower range than corn and probably reaches its upper limit near the figures given.

There are but three allowable causes in the present state of our knowledge, to which might be attributed the slackening or inhibition of growth or actual shrinkage of growing joints after midday and continuing until the following morning. The retardations in question are relatively least in the earlier stages of development when the joints are not more than one fourth or one fifth adult size and

have the effect of a flattening of the curve that is of slowing down growth. The action becomes more pronounced until a stage is reached when more and more of the elongation of the forenoon is retracted in the afternoon (see Fig. 3).

Such negative action might be due to the reduction of an enzyme concerned in the renewal of the constructive material below the effective amount, or to the clogging action of accumulated products, or as has been previously suggested, to transpiration counterbalancing imbibition and accretion of suspended material. Cessation of growth at 26° C. to 30° C. would be difficult to reconcile with the assumption that it might be due to a destruction of an enzyme, since all known bodies of this kind do not begin to show a rapid rate of disintegration until a much higher temperature is reached. An accumulation of the products in some part of the chain of reactions might well take place, however. Similar retardations in photosynthesis are known to occur when translocation of the carbohydrates is prevented.

As to the third suggestion it is to be said that the stoppage or slackened growth of green plants in the open in the hours immediately preceding daylight coincides with a condition of lessened imbibition capacity due to high acidity and accompanied by the most rapid transpiration displayed by the plant. The low temperatures at this time might also cause a decreased absorption. The rate of absorption of green plants would be greatest in the afternoon, and as water-loss at this time has been found to be actually less than in early morning, it is to be seen that the decreased growth characteristic of this part of the day may not be attributed to excessive transpiration. Acidity is near the minimum at this time and the imbibition capacity of the growing joint is greatest. That transpiration may actually check or neutralize growth has been demonstrated in *Eriogonum* by Lloyd:⁹

The daily march of growth is as follows: During the early daylight hours until about 8, there is usually a slight rise in growth rate. After that hour the rate falls to a low value, or, much more frequently there ensues an actual shrinkage. This is the period during which the loss of water by transpiration is rapidly increasing, reaching its maximum at about noon. Coincidentally with the checking of transpiration, the growth rates rapidly increase in value.

⁹ Report Dept. Bot. Research, Carnegie Inst. of Washington for 1916.

the maximum rate being attained by 1 or 2 P.M. and thereafter maintained, with fluctuations, until 6 P.M., when the rates again fall to the night values. The afternoon rates are great enough to more than make up for the negative behavior of the morning, except, as above stated, under unusual conditions.

That light cannot be held to account for the retardation of growth during the morning hours as above indicated has been shown to be an untenable view, since it was found possible experimentally to alter the rates both positively and negatively quite independently of the constancy, increase or decrease of illumination, even when this has been increased with respect to the growing part by insolation from three directions. There seems indeed to be no maximum insolation normally occurring in the field at this locality which can cause any cessation or inhibition of growth when conditions which insure water supply to the growing part obtain. Thus, when a cessation of growth is apparent, it can be checked, and high rates instituted, by the removal of leaves (which divert the water supply), by increasing the vapor tension in the vicinity of the growing part, or by merely increasing the temperature when the volume of the growing part is small (as when the internode under observation is young). These positive changes may occur coincidentally with increase of illumination from the blue or red portions of the spectrum to full insolation.

A similar action may occur in the inactivity of green opuntias in the open, but certainly does not apply to the daylight retardation. On the other hand the checking of growth or shrinkage of etiolated members in darkness and of green shoots at high temperatures may well be due to transpiration or modification of imbibition capacity.

WATER-ABSORBING CAPACITY OF PLANT TISSUES.

Growth is essentially the irreversible enlargement of embryonic cells, by the appropriation of material of which 98 or 99 per cent. is water. The process depends upon the availability of the building material which enters into the structure of the protoplast, its inclusions and its envelopes, and upon the continuance of reactions, such as enzymosis and respiration, which maintain an unsatisfied absorptive capacity.

The incorporation of the solutions in the colloids of the protoplast is essentially a hydration process which is usually designated as imbibition. A stable colloid takes up a fixed solution at a rate expressible by a regular curve. The protoplast is a complex mixture of both emulsoids and suspensoids in which there is almost unceasing change. Its structure may be modified by the uneven action of the metabolic plexus which may also result in the accumulation of

products such as acids, the presence of which may cause acceleration, retardation or cessation of growth by modifying imbibition or capacity for absorption of water.

It is obvious that a determination of the water-absorbing capacity or swelling coefficient of a growing organ would be an index of its capacity for enlargement at that moment, and by the use of differential solutions the influence of acidity or alkalinity on the process may also be ascertained. The catabolic and synthetic processes which accompany growth are in the main continued in mature organs, especially if these contain tracts of open meristem as do the joints of *Opuntia*. It was thought highly important therefore to make extensive tests of the swelling capacity of *Opuntia* with analyses of the carbohydrate content of the joints. These tests yield some data of great interest when considered in connection with the growth records given in the preceding section of this paper.

The flattened joints of *Opuntia sp.* which formed the principal experimental material are elongated oval in outline, the basal part being usually about 20–24 mm. in thickness and the apical part half or less than half of this diameter. After some extensive comparisons of sections from all parts of the joint it was found that the apical third of the member furnished the best material for comparative purposes. Sections or disks about 12 to 14 mm. across were cut from this region with a cork borer, avoiding the inclusion of nodes bearing the spines and spicules. Such sections consisted of the indurated epidermal layers between which was a cylindrical mass of parenchymatous cells, the outer ones being chlorophyllous and some of the inner ones being mucilaginous. An anastomosed network of thin fibrovascular strands was included in the parenchymatous mass and this mechanical tissue probably checked expansion in some cases, especially those in which disks were taken too close to the nodes. More care was exercised in this matter in 1917 than in the preceding tests, a fact that may be taken to explain in part at least the decreasing number of anomalies as the work progressed. Three of such disks about 12 mm. across the epidermal surfaces and from 6 to 11 mm. in thickness were arranged in a triangle in the bottom of a stender dish and a triangle of thin sheet

glass arranged to rest its apices on the three disks. The vertical swinging arm of an auxograph was now adjusted to a shallow socket in the center of the glass triangle while the pen was set at zero on the recording sheet. Water or a solution being poured into the dish, the course of the swelling was traced, the record showing the averaged result of the action of the trio of specimens (see Fig. 7). That the amount of imbibition depended upon the presence of certain recognizable substances was demonstrated by the fact that dried

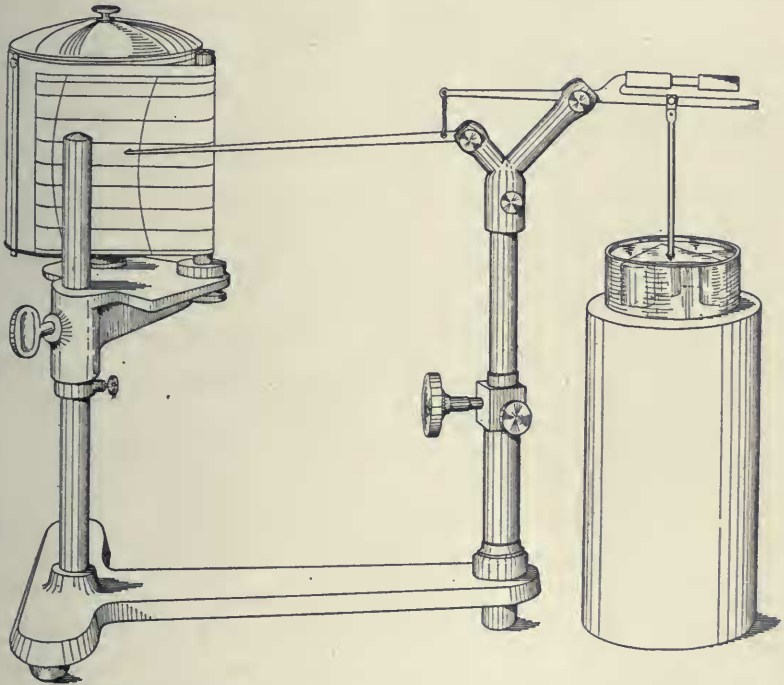


FIG. 7. Auxograph arranged for recording changes in thickness of trio of cylindrical sections of *Opuntia*. The vertical arm, which is set in position on horizontal arm to give a magnification of twenty, rests on a triangle of glass laid on top of the sections. The dish containing the sections rests on an iron cylinder to secure stability and a weight is placed on the T base of the instrument. The record sheet is ruled to millimeters (not shown) with heavier horizontal lines 1 cm. apart. The heavy curved lines shown represent four hour intervals. The space is ruled to fifteen minute intervals (not shown). Height of clock and lever supports adjustable.

and dead disks gave proportionate differences equivalent to those shown by freshly cut and living material.

The auxograph used in making the measurements represents an modified form of an apparatus originally designed by the senior author in 1901. The improved instrument consists of a compound lever, the components of which are suspended in adjustable bearings held in the arms of a metal support of "Y" form with the arms of unequal length. One free arm of the bearing lever is forked, the upper segment carrying a counterpoise which may be moved to give any desired pressure on the bearing contact with an object the swelling of which is to be measured. The lower segment of the free part of the bearing lever has a sleeve with a short socket hinged to its lower side. A thin glass rod set in this socket extends downward to a length of a few centimeters and rests in a concavity in the center of a glass plate laid on the trio of sections in a suitable small glass dish. The sleeve may be moved along the lever to give a magnification between ten and fifty to a pen carried by the other free lever arm. The two small levers are connected by a short length of jewelers' chain in such manner as to minimize friction and other sources of error. The pen is arranged to bear on a slip of paper 8 cm. wide ruled to millimeters and it is carried by a cylindrical clock which gives it a movement of 28 cm. in 24 hours. The compound lever was supported by a rack and pinion column which made it adjustable through a range of 12 cm. in height.

The clock may be moved vertically on its support and fastened at any height by a set screw. The delicacy of this apparatus was such that it could not be operated on a wooden table in an ordinary room. Cement, stone or brick piers with a slab of slate, wood or stone furnished the necessary steadiness. The dishes in which the sections were immersed in swelling solutions were placed on top of iron cylinders 15 cm. high and about 8 cm. in thickness and the dishes were held in place by clay luting. A weight of about 4 or 5 kg. placed on the "T" base of the instrument completed an arrangement by which it was possible to secure undisturbed records of swelling of sections of cactus, of plates of colloids, and also of growth of joints of this and other plants.

The following measurements of the swelling capacity of sections from the terminal joints were secured in 1916 and 1917. One

set was made from joints which had been formed during the previous year. Their development as buds began in March and April and was nearly complete by June 1st. Some enlargement may ensue later in the season, or in the following season, as has already been described.¹⁰

SWELLING—*Opuntia Sp.*

Mature Joints.

(See Fig. 8.)

(Joints of 1915.)

	Water Percentage.	HCl N/100 Percentage.	NaOH N/100 Percentage.
May 19, 1916	50.0	43.3	70.0
" 26, "	40.0	36.6	52.1
June 3, "	72.2	35.3	72.6
" 13, "	23.9	53.6	55.1
" 10, "	51.7	35.7	57.6
Nov. 25, "	65.0	62.0	54.1
(Measured)	47.6	50.0	35.5
Jan. 28-30, 1917	37.6	34.3	36.0
Feb. 20-21, "	12.3	9.1	10.3
" 23-24, "	14.7	19.9	19.1
Mar. 27-28, "	11.0	10.9	11.0

Swelling of Other Joints Three Years Old.

	Water. Per Cent.	HCl N/100. Per Cent.	NaOH N/100. Per Cent.
May 23, 1916	54.4	40.4	58.5
Dried disks of percentage of original diam. ...	41.3	31.6	42.4

The swelling capacity of sections appears to increase with development and rising temperatures to June at which high values were shown by both young and mature joints. A decrease during midsummer is followed by a maximum reached in November.

The average swelling of young joints was 31.2 per cent. in water, 28.9 per cent. in acid and 29.5 per cent in alkali for the season.

The variations in swelling capacity during the second year are indefinite but an average of the available records (seven tests) shows 50.5 per cent. in distilled water, 45.2 per cent. in hundredth normal hydrochloric acid and 56.7 per cent. in hundredth normal

¹⁰ MacDougal, "Mechanism and Conditions of Growth," *Mem. N. Y. Bot. Garden*, 6: 5, 1916.

Swelling of Joints Formed in 1916.

(See Fig. 8.)

	Water. Per Cent.	HCl N/100. Per Cent.	NaOH N/100. Per Cent.
May 18, 1916	24.3	30.0	40.0
June 2, "	23.6	16.4	22.9
" 13, "	70.1	41.5	49.1
Aug. 3, " (swelled at Carmel)	16.6	14.0	14.3
" 3, " (grown and swelled at Carmel)	18.2	9.3	15.7
Nov. 2, "	20.5	21.0	22.2
" 3, "	14.6	21.3	19.5
" 4, "	28.0	28.0	28.3
" 5, "	27.9	26.0	24.7
" 6, "	20.8	18.4	17.1
" 6, "	27.9	26.0	24.7
" 23, "	44.0	53.5	46.0
" 23, "	34.4	34.9	35.3
" 23, "	49.3	47.9	47.0
" 23, "	48.0	45.4	35.3
Jan. 24-25, 1917 (12 sections)	25.7	27.9	25.0
Feb. 20-21, " (6 ")	10.7	11.7	10.8
Mar. 23-24, " (6 ")	9.4	12.0	10.9
April 24	21.8	20.4	13.9
	20.4	21.8	33.8

sodium hydrate. Inspection of the data obtained by the chemical analyses fails to bring to light any connection between the amount of imbibition and the proportion of any carbohydrate or salt present. The diverging variations suggest combinations of substances to which the swelling may be due. It is to be noted that the proportionate swelling of the sections would be lowered by the thickness of the sections which are fifty to seventy times the diameter of the colloid sections used in other experiments. Furthermore, the amount of swelling is in all probability lessened by the presence of mechanically resistant fibrovascular tissue.

IMBIBITION AND CARBOHYDRATE METABOLISM.

In the foregoing pages special attention has been directed to the conditions affecting imbibition and the water-absorbing capacity of the growing plant cell. It is evident that the metabolic activity of the cell itself affects imbibition very greatly; an accumulation of the intermediate or end products of respiration may thus cause an in-

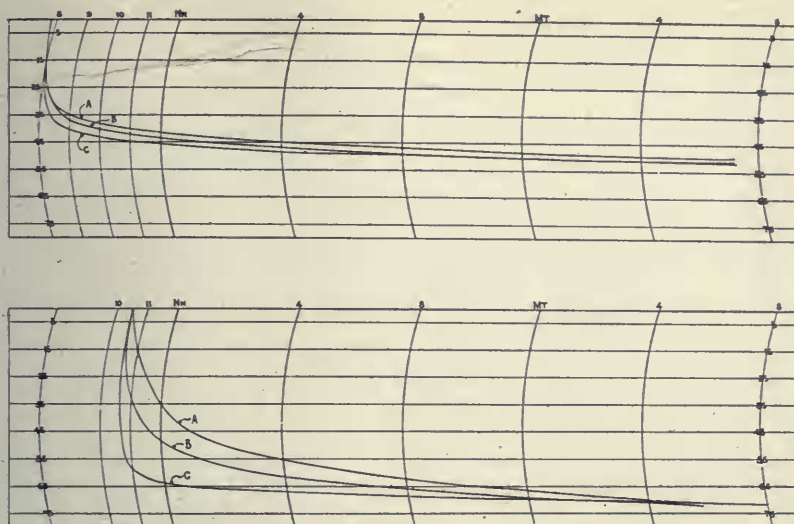


FIG. 8. Auxographic tracings of swelling of cylindrical sections of *Opuntia Sp.*—joints formed in 1915. A compound lever set to magnify swelling 20 times carries a pen downward from the zero line on a sheet 80 millimeters in width, carried past the pen in 24 hours. The right hand or upper line *a* was traced by a trio of sections of an average diameter of 13 mm. which showed a swelling of $50/20 = 2.5$ mm. in hundredth-normal sodium hydrate, which was 19.2 per cent. of the original. The lower line *c* was traced by a trio of sections of an average diameter of 13.8 mm. in hundredth-normal hydrochloric acid which showed a swelling of 2.55 mm. or 18.5 per cent. The middle line was traced by a trio of sections of an average diameter of 12 mm. which swelling 2.55 mm. or 21.3 per cent. Feb. 22, 1917. (Upper half of figure.) Reduced $\frac{1}{2}$.

Auxographic tracings of old joint of *Opuntia blakeana*. The upper right hand line *a* was traced by swelling of trio of sections of an average diameter of 10 mm. in hundredth-normal sodium hydrate. The increase was 3.6 mm. or 36 per cent. The middle line *b* was traced by the swelling of a trio of sections of an average diameter of 11 mm. in distilled water. The swelling was 3.6 mm. or 32 per cent. The lower line *c* was traced by the swelling of a trio of sections with an average diameter of 10 mm. in distilled water. The swelling was 3.5 mm. or 35 per cent. of the original. A notable difference between the rates of swelling in the three solutions is exhibited in contrast with those of the series of joints of 1915. (Lower half of figure.) Reduced $\frac{1}{2}$.

crease or decrease in the water-absorbing capacity of the colloidal substratum of the cell. At the same time the degree of imbibition and of swelling plays an exceedingly important part in metabolism and hence in the formation of plastic material necessary for growth

and in the liberation of energy. Although these two activities, imbibition and metabolism, are so closely interrelated in the growth processes they are nevertheless of such a widely different nature that it cannot be assumed, as will be shown, that they are equally influenced by external conditions, as for instance, temperature; the conditions under which one affects the other depending, in turn, upon several other factors.

In general, chemical inversion, or the transformation of the highly condensed to the simpler molecules capable of oxidation and translocation, takes place only under conditions of ample water supply. However, these reversible enzymatic reactions never run entirely in one direction. Only differences between the two are observable. We are dealing with a delicate compound dynamic equilibrium, involving probably dozens of steps and many more substances. The very interesting investigations of Lobry de Bruyn and Van Ekenstein¹¹ and of Nef on the rearrangements of the hexose molecule demonstrate the extreme complexity of such equilibria. Thus Nef¹² has shown that when the relatively simple hexose sugar, dextrose, is dissolved in a weak alkaline solution there are formed no less than 93 different substances which constitute a system in dynamic equilibrium. Any number of these can react selectively and shift the equilibrium, by oxidation, condensation or the like, the course of the reaction depending upon the condition of solution as to concentration, temperature, etc. How much more complex must the condition be in the living cell with the numerous delicate enzymatic equilibria each with its own temperature and concentration coefficient?

The following results (which are a portion of an extensive investigation of the carbohydrate economy of cacti now in progress) throw some light on the relation of carbohydrate metabolism to growth.

The carbohydrates predominate in the general food economy of the cacti. There is no reason for believing that the metabolic processes concerned in the growth of such plants consist chiefly of

¹¹ Lobry de Bruyn and Van Ekenstein, *Rec. trav. chim. de Pays-Bas*, 14, 158, 203; 15, 92; 16, 257.

¹² Nef, J. U., *Annalen der Chemie, Liebig*, 403, 204-383, 1913.

protein synthesis and catabolism as is probably the case in animals. In fact these plants behave largely like masses of gel of carbohydrate nature.

Roughly the fresh material of the growing and mature joints is composed of about :

	Per Cent.	Per Cent.
Water	95	75
Crude protein	0.5	1.0
Carbohydrates hydrolyzable with 1.0 per cent. HCl	5.0	10.0
Cellulose	1.0	3.0
Crude fat	0.25	0.5
Ash	1.0	3.5

The total carbohydrate content and of food supply in general is of little significance or value in studying the various functions of an organism such as the cactus. It is rather the nature of the sugars, or the degree of general chemical inversion, that determines the supply of building material necessary for growth. The records show many instances of large food supply, and all known external conditions favorable for growth, and still no such action taking place. The question of rest period undoubtedly is largely one of adjustment of chemical inversion and reversion, and in general the conditions favoring the awakening of buds are those in which inversion has attained a lead over reversion, permitting a sufficient accumulation of plastic material; while on the other hand, an accumulation in the protoplasmic medium of the products of reversion affects the inhibiting of growth. It seems therefore that in order for growth to occur there must be a sufficient supply of the simpler sugars necessary for respiration as well as for the synthesis of new substances, that synthesis can overbalance the break-down with the accumulation of new material, the latter being the product of an irreversible reaction. In the study of the relation of carbohydrates to growth it is therefore a question of the carbohydrate balance, the ratio of the simple to the condensed sugars that is of prime importance.

The problem of determining the different sugars in a growing organism is one of great difficulty because, as has been indicated, of the large number of sugars belonging to the same group and of the similarity of their chemical properties. It must therefore suffice to

determine together groups of sugars of the same general physiological significance. It has been found preferable for the present to make a large number of analyses with as great accuracy as possible, rather than attempt to isolate and determine each of the sugars in a few cases, especially as individual cases show considerable variation. For the present purpose a discussion of the methods of analysis¹³ employed does not seem essential.

The following experiment will illustrate the effect of water on the carbohydrate balance of *Opuntia discata*. A number of joints of the same age were taken from one plant and divided into three lots each of six joints. The first (1) was analyzed immediately, the second (2) was suspended in battery jars without water, and the third (3) was placed in the same manner in battery jars so that the base of the joints were immersed as in a water-culture. (2) and (3) were kept in a dark constant temperature room at 28° for thirty days, when they were analyzed. The joints in water had developed roots 5 to 10 cm. in length.

	Immediate (1).		Dry (2).		Water (3).	
	Fresh.	Dry.	Fresh.	Dry.	Fresh.	Dry.
Water	80.34		77.20		82.30	
Total sugars	4.30	20.49	4.29	18.84	3.60	18.58
Total polysaccharides	3.50	17.80	3.60	18.01	2.80	17.54
Hexose-polysaccharides	1.65	8.40	1.81	8.83	1.25	7.85
Disaccharides and hexoses	0.10	0.49	0.13	0.56	0.14	0.83
Disaccharides	0.04	0.20	0.07	0.30	0.06	0.38
Hexoses	0.06	0.29	0.06	0.26	0.08	0.45
Pentosan	1.74	8.86	1.78	9.18	1.25	7.85

The joints without water (2) lost 3.14 per cent. in water content, while those in water (3) gained 1.96 per cent. In total polysaccharides and hexose-polysaccharides (3) is considerably lower than (2), while in hexoses (3) shows a gain over (1) and (2).

The difference in the carbohydrate balance between plants growing in the desert and in Carmel, California, is illustrated in the following analyses of *Opuntia sp.* during September. The values are per cent. of fresh weight:

¹³ Full particulars thereof will appear in a later publication on the "Carbohydrate Economy of Cacti."

	Carmel.	Tucson.
Water	91.15	80.34
Total sugars	2.61	4.30
Total polysaccharides	1.94	3.50
Hexose polysaccharides09	1.65
Disaccharides07	0.04
Hexoses52	0.06
Pentoses14	0.05
Pentosan	1.70	1.74

Under natural condition similar relations exist. The following table gives typical results of a large number of analyses of *Opuntia* *sp.* made during each month:

Date.	March 7.	April 3.	April 18.	May 5.	June 9.	July 3.	July 31.	Sept. 20.	Oct. 26.	Nov. 15.	Dec.
Dry weight.....	15.25	18.20	18.90	21.30	26.74	30.32	16.45	19.66	20.3	23.05	30.1
Total sugars.....	3.49	4.11	5.58	4.81	6.52	5.07	2.42	4.30	4.24	4.80	5.70
Polysaccharides.....	2.80	3.13	4.70	4.55	6.31	4.92	2.26	4.24	4.06	4.40	5.25
Monosaccharides....	0.69	0.98	0.88	0.26	0.21	0.15	0.16	0.11	0.18	0.40	0.45

Naturally conditions are somewhat more complicated than those in the tests described on p. 336. At the time the new shoots begin to grow, during the end of March and early April, after the winter rains, the parent joints have a high monosaccharide content. As the dry summer advances the amount of these sugars diminishes, although the total sugars increase. With the advent of the summer rains, at the end of July, the decrease in monosaccharides is checked though the high temperatures and resulting high rate of respiration does not permit an accumulation. Another factor entering here is the effect of the temperature on the enzymatic equilibrium. Separate experiments have shown that at the temperatures which prevail in the cacti at this time (during the day as high as 55° C.) there is a distinct shifting in favor of the polysaccharides. During the dry months of September and October the monosaccharides drop to a minimum, in spite of the temperature being considerably lower. With the winter rains there is again an accumulation which is maintained during the winter until spring, when the favorable temperatures again permit growth. The formation of new shoots does not take place in spring when an accumulation of monosaccharides has

been prevented, for instance, by means of keeping the joints at a raised temperature in the light during the winter time. However, it need hardly be emphasized that the supply of simple sugars can not be regarded as a single determining factor for growth or the awakening of buds. Such material is essential for the construction of new cells, but as yet no definite conclusions can be drawn as to the exact physiological rôle of the various hexoses and pentoses. When the joints are subjected to starvation, *i. e.*, are placed in the dark for periods of from one to nine months, these simple sugars are used up more rapidly than they are formed from the relatively large store of polysaccharides. With the decrease of the supply of monosaccharides the accumulated organic acids, intermediate products of the normal respiration, are drawn into the process and the total acidity of the organism is thus reduced. Reduced acidity is accompanied by an increased imbibition of the cactus in water. It is also highly probable that other intermediate and end products of metabolism that accumulate in the colloidal substratum of the cell, and affect imbibition as will be shown in the next chapter of this paper, are also removed, resulting in the same effect on the water-absorbing capacity as the removal of the organic acids. Thus cactus joints with a swelling capacity of 20 per cent. in water after being starved four months were neutral to litmus indicator and showed a swelling of 100 per cent. During this period the dry weight of the cactus remained the same.

It is as yet impossible to determine definitely the carbohydrates which make up the colloidal substratum of the cactus cells. Theoretical considerations would require that these be substances of relatively slight physiological reactivity, *i. e.*, substances which are not utilized in the course of metabolism as sources of energy, and are little susceptible to enzymatic disintegration. Of special importance in this connection are the unfermentable sugars which have been found to be present in relatively large amounts, mostly in the condensed form as pentosans.

THE BEHAVIOR OF CARBOHYDRATES AND PROTEINS IN GELS USEFUL
IN THE INTERPRETATION OF THE ACTION OF PLANTS.

The amorphous carbohydrates constitute a very important part of the colloids of the protoplast, the remainder of which consists largely of nitrogenous material, in the form of albumen or albumen derivatives with an unknown amount of lipin. The search for material which might simulate the imbibitional behavior of growing tracts in plants begun by the senior author resulted in finding that mixtures of agar with gelatine in which the last-named substance was present in the smaller proportion showed an enhanced capacity for imbibition in distilled water and a reduced swelling in weak acid and alkali as measured in very thin plates by the auxograph.¹⁴

The swelling of gelatine in percentages of the original thickness of thin dried layers or plates (.1 to .3 mm. in thickness) in water, hydrochloric acid and sodium hydrate, may be illustrated by the following data which represent averages of measurement at the

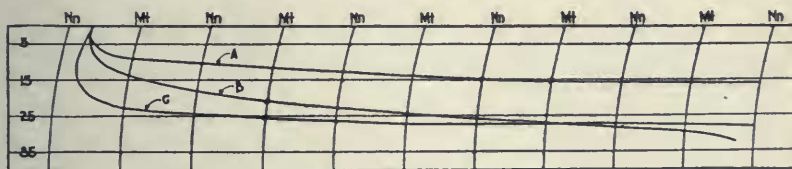


FIG. 9. Auxographic tracing of swelling of agar sections .2 mm. in thickness in NaOH N/100, A = 400 per cent., in HCl N/100, B = 650 per cent., and in distilled water, C = 775 per cent. $\times 10$.

end of sixteen hours (see p. 343 for further discussion of swelling determinations by use of thin plates).

Water.	HCl N/100.	NaO N/100.
471.5 per cent.	1012.3 per cent.	587.5 per cent.

Similar plates of agar gave swellings as follows (Fig. 9) :

Water.	HCl N/100.	NaO N/100.
462.5 per cent.	725 per cent.	937.5 per cent.

¹⁴ MacDougal, "Imbibitional Swelling of Plants and Colloidal Mixtures," *Science*, N. S., Vol. 44, No. 1136, pp. 502-505, October 6, 1916. See also Ann. Report, Dept. Bot. Res., Carnegie Institution of Washington for 1916, pp. 61-64.

As the plant did not show water relations which might be interpreted as a direct combination of the separate action of gelatine or agar, it was next proposed to test the reactions of a mixture in which these substances would be blended, which was done in July, 1916. The first test mass was one consisting of about equal parts of agar and gelatine, though the quantities were not weighed. Both were soaked and melted separately and the gelatine was poured into the hot agar which was kept at a temperature of about 90° C. for a half hour. The mass was then poured onto a glass slab for cooling. Two days later it was stripped off as a fairly clear and transparent sheet slightly clouded, the average thickness of which was 0.2 mm. Strips about 5 × 7 mm. were placed under the apices of sheet glass triangles in glass dishes after the manner in which plant sections had been tested, and auxographs were arranged to record the action of acids, alkalies, and distilled water. This mixture gave swellings as follows:

Water.	HCl N/100.	NaO N/100.
762.5 per cent.	687.5 per cent.	800 per cent.

The mixture of these two substances having been found to swell more in water and in alkaline solutions than in acid, a series of varying proportions of the two constituents were made up. The mixtures were poured into moulds on glass plates and dried sheets from .1 mm. to .6 mm. in thickness were obtained. The measurements given below include the averages of tests under varied conditions not only of thickness of the samples, but also of temperature, length of period of swelling, tension of instruments, etc. The principal results obtained were as follows:

<i>Gelatine 100—Agar 1.</i>		
Water.	HCl N/100.	NaOH N/100.
750 per cent.	1100 per cent.	520 per cent.
<i>Gelatine 100—Agar 5.</i>		
329	850	685.5
<i>Gelatine 80—Agar 20.</i>		
431.6	789.3	760.7
<i>Gelatine 50—Agar 50.</i>		
799.0	366.6	580.9

Water.	HCl N/100.	NaOH N/100.
378.0	Gelatine 25—Agar 75. 427.3	510.7
1144.5	Gelatine 20—Agar 80. 572.1	526.0
1000.0	Gelatine 10—Agar 90. 401.0	300.0
1825.0	Gelatine 1—Agar 99. 475.0	425.0

The data indicate that as the proportion of agar in the mixture is increased, the relative amplitude of swelling in water may be increased, and the relative amount of imbibition in acid is decreased. This superior imbibition capacity in water as compared to effects of acid and alkali is a fair parallel to the behavior of sections of young, mature and old parts of *Opuntia*.

The second parallel of importance is the one in which the swelling in alkaline solutions is in some cases less and in others greater than in acidified solutions in mixtures containing as much as a third or more of agar.

The mucilaginous material which may be obtained by macerating joints of cacti in distilled water is fairly similar to agar. Some of this was used in mixtures in place of agar. The averages of a series of swellings of a mixture of 90 parts of gelatine and 10 parts of such mucilage, reckoned by dry weight, were as follows:

Water.	HCl N/100.	NaOH N/100.
428.1 per cent.	770.4 per cent.	557.8 per cent.

These data are of interest when compared with the swellings of mixtures of 100 parts gelatine to 5 parts agar, and of mixtures of 80 parts of gelatine to 20 parts of agar (see p. 340). The mucilage from joints of *Opuntia* affects the swelling of gelatine in much the same manner as does agar in equivalent proportions. The watery extract of course contains the soluble salines of the plant, and some of the effect might be attributed to their presence.

A few simple tests were arranged to show the effects of a salt on the colloids used, the results of which are as follows:

GELATINE.

Swelling.

	Water.	HCl N/100.	$\frac{\text{HCl} + \text{NaCl}}{\text{N}/200}$
	450.0 per cent.	1200.0 per cent.	1116.7 per cent.
	516.7	1066.7	1400.0
	—	—	1250.0
Averages:	$\frac{483.4}{}$	$\frac{1133.4}{}$	$\frac{1255.6}{}$

Gelatine.

	Water.	HCl N/100.	HCl N/100 + NaCl N/100.
	616.7 per cent.	1016.7 per cent.	833.3 per cent.
	466.7	1083.3	1083.3
	—	1133.3	883.3
	—	—	866.7
	—	—	833.3
Averages:	$\frac{483.3}{}$	$\frac{1077.8}{}$	$\frac{899.9}{}$

The superior swelling of gelatine in acidified solutions is illustrated and a lower average of swelling in hundredth normal hydrochloric acid in the presence of a salt solution of the same concentration was demonstrated. The admixture of hundredth normal acid and of hundredth normal salt solution gives a solution of two hundredths normal concentration. Gelatine shows a lesser swelling in this weaker acid, and furthermore the presence of the salt appears to increase imbibition.

Sugars are an important constituent of living tissues and it is highly probable that in addition to pentose, sucrose and dextrose are also in the colloidal suspensions of the protoplast. It was important to determine whether or not they exerted any direct effect in the concentrations in which they might occur in the cell. A series of tests of the effects of these substances was carried out by Mr. E. E. Free at the Coastal Laboratory in September, 1916. Gelatine and agar were mixed in various proportions, dried to thin sheets and then swelled at temperatures of 16 to 21° C.

Sugar solutions of a concentration less than 25 per cent. did not differ appreciably in its effects from distilled water. Sucrose concentrations of a 50 per cent. concentration produced a markedly lessened concentration of all gels. Dextrose of the same strength

had a similar effect on the mixtures low in gelatine in which it was tried. Its effect on mixtures containing a large proportion of gelatine was not determined. The appreciable effects are probably due to the tying up of molecules of water analogous to the osmotic action of such solutions.

Sugar solutions of a concentration of 25 per cent. or higher are not characteristic of growing regions and probably occur only in storage tracts, seeds or cotyledons. While the effect would be to lessen imbibition by the colloidal mass of the protoplast it is to be recalled that a vacuolar fluid of such concentrations would have high osmotic properties and the expansion by turgidity might mask or exceed that due to imbibitional swelling. If sugars contribute directly to the growth expansion of the cell it would therefore be in the later stages of development and by osmotic action.

A duplicate series of tests of the behavior of an admixture of starch with agar gave the following results:

SWELLING.		
<i>Agar 90—Starch 10.</i>		
Water.	HCl N/100.	NaOH N/100.
1275 per cent.	541.6 per cent.	496.6 per cent.

The complication of the carbohydrate gel by the addition of starch made no essential departure from the behavior of agar alone in water, acidified and alkaline solutions.

The combination of agar and gelatine gave a gel in which two of the three main groups of constituents of living matter were represented.

It is not certain, however, that the combination of amino-acids in gelatine is duplicated in the plant and it was deemed important to test the effects of simpler amino-acid compounds and of the more complex albumens on the swelling of agar, as representing the basically important carbohydrates. Solutions of the various mixtures were poured on glass plates in layers about a centimeter thick and 3 by 5 cm. in area. Desiccation resulted in a reduction of the length and width to about half of the original. The thickness however was reduced to one-tenth or even as much as to one-thirtieth of the original, and having a thickness of .1 mm. to .3 mm. in most

cases. The principal axis of deposition of material was in the vertical and the swelling in this direction would of course be correspondingly in excess of that in the plane of the sections. It is extremely unlikely that any of the colloidal masses of the cell are iso-radial as to deposition or structure and the use of thin plates seemed a feature which might increase the similarity of behavior with that of the plant. The strands, sheets or masses of material in the cell are of course mostly thinner than the plates used in the experiments, which however would affect speed of imbibition more than final proportion.

Trios of sections of sheets of the dried colloids 2 to 4 mm. by 3 to 6 mm. were placed in the bottom of stender dishes or of heavy watch glasses securely seated on iron cylinders. Triangles of glass were placed on the sections, and the vertical arms of auxographs were rested in a socket in the center of the triangles. Any change in thickness of the sections would be registered immediately. The use of six instruments gave duplicate results of the effects of water, acid and alkali, and each record was an integration or average of the swelling of three sections.

The only albumen available when this plan was put into operation was a commercial egg-albumen, and this was first tested in mixtures with large proportions of gelatine. The results of the swellings are as follows:

Water.	HCl N/100. Gelatine.	NaOH N/100.
	(Average of 3 tests.)	
313.8 per cent.	825.5 per cent.	558.3 per cent.
	<i>Gelatine 100—Albumen 5.</i>	
	(Average of 5 tests.)	
283.4	611.7	482.2
	<i>Gelatine 85—Albumen 15.</i>	
	(Average of 5 tests.)	
408.6	827.8	673.0
	<i>Gelatine 75—Albumen 25.</i>	
	(Average of 3 tests.)	
378.3	569.7	508.7

The albumen did not exert any important influence on the swelling of the mixture until it was present in proportions as great as 25 per cent. The action is not marked even in this high proportion. Neither this nor any other combination in which gelatine formed the greater part displayed water relations at all similar to those of the plant.

Next egg-albumen was added to agar and agar-gelatine mixtures with results as below, a further illustrative test being made of agar-gelatine:

Water.	HCl N/100.	NaOH N/100.
	<i>Agar 75—Gelatine 25.</i> (Average of 4 tests.)	
378.5 per cent.	427.3 per cent.	515.7 per cent.
	<i>Agar 90—Albumen 10.</i> (Average of 3 tests.)	
1516.6	270.0	333.3
	(Average of 6 tests.)	
1477.1	309.8	297.9
	<i>Agar 70—Gelatine 20—Albumen 10.</i>	
595.0	216.6	298.6

The addition of ten per cent. of albumen to agar notably reduced the capacity of agar for swelling in acid and alkali, and appeared to increase the amplitude of swelling in distilled water, although the last matter is not entirely clear. The albumen reduced the swelling of a mixture containing twenty-five per cent. of gelatine slightly in acid and in alkali, but the swelling in water was not markedly greater. This preliminary test yielded results which made their extension highly desirable. Chemical analyses of the egg-albumen were not available, and as nothing was known as to the salts or other substances which might be included, it was desirable to secure material of known origin and composition. Arrangements were made with Dr. Isaac F. Harris, of Squibb and Sons Laboratory, New Brunswick, New Jersey, to prepare some albumen from beans (*Phaseolus*) and from oats (*Avena*) to be used in the mixtures. The preparations from *Phaseolus* were available in February, 1917, and the first tests were made with the "protein" extract which contained the water soluble salts of the bean and the proteins which were soluble in water containing these salts.

Agar and gelatine were dissolved in the usual way and the temperature of the suspension allowed to fall to a point below 40° C. before the protein was stirred into it. In the course of the cooling and drying, cloudy masses became visible which were taken to be the globulin component of the protein. The dried sheets came down to a thickness of .3 to .4 mm. Calibrated samples were tested in trios under the auxograph in the usual manner. Two complete series of

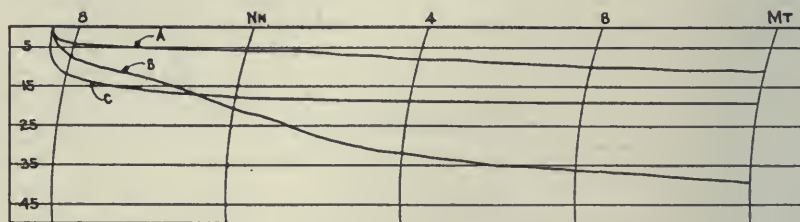


FIG. 10. Auxographic record of swelling of agar 90—protein 10, sections .25 mm. in thickness, in NaOH $N/100$, $A = 220$ per cent., in HCl $N/100$, $B = 360$ per cent., and in distilled water, $C = 800$ per cent. $\times 10$.

all mixtures were made and an additional measurement of the action of water and alkali was obtained. The swellings were as follows (Fig. 10):

	Water.	HCl $N/100$.	NaOH $N/100$.
<i>Gelatine 90—Protein 10 (Phaseolus).</i>			
	585.7 per cent.	1401.0 per cent.	942.8 per cent.
	486.0	1200.0	704.3
	386.0	—	800.0
Averages:	$\frac{485.9}{}$	$\frac{1300.5}{}$	$\frac{817.7}{}$

	Water.	HCl $N/100$.	NaOH $N/100$.
<i>Gelatine 75—Protein 25 (Phaseolus).</i>			
	696.9	818.1	621.2
	500.0	1060.6	848.4
Averages:	$\frac{598.5}{}$	$\frac{939.4}{}$	$\frac{734.8}{}$

	Water.	HCl $N/100$.	NaOH $N/100$.
<i>Agar 90—Protein 10 (Phaseolus).</i>			
	800.0	50.0	150.0
	800.0	75.0	150.0
Averages:	$\frac{800.0}{}$	$\frac{62.5}{}$	$\frac{150.0}{}$

	Water.	HCl $N/100$.	NaOH $N/100$.
<i>Agar 99—Protein 1 (Phaseolus).</i>			
	1080.0	300.0	220.0
	800.0	360.0	240.0
Averages:	$\frac{940.0}{}$	$\frac{330.0}{}$	$\frac{230.0}{}$

The protein extract from the bean was thus shown to exert an influence on the swelling of agar similar to that of egg-albumen in reducing the amount of swelling in acid and alkali, and increasing it in distilled water.

The next step of importance was to ascertain the effect of some of the simpler amino-acids which might be derived from the albumens in the plant. Tyrosin and cystin were available. As an example of the method the first preparation of tyrosin was one in which one part of this substance in solution was stirred to a liquefied mass of ten parts of agar at a temperature of 32° C. This was poured on a glass slab, and as desiccation was carried out the tyrosin began to collect as a flour-like efflorescence on the surface, and apparently a large part of the substance came out in this way, so that the actual



FIG. 11. Auxographic record of swelling of sections of agar 90—tyrosin 10, .15 mm. in thickness, in NaOH $N/100$, $A = 133$ per cent., in HCl $N/100$, $B = 233$ per cent., and in distilled water, $C = 1600$ per cent. $\times 6$.

proportion of the amino-acid in the dried plate was probably not more than a fourth of the amount originally used.

The dried plate of material came down to a thickness of .15 mm. and gave the following results (Fig. 11):

SWELLING.

Agar 90—Tyrosin 10 (less by efflorescence).

Water.	HCl $N/100$.	NaOH $N/100$.
1600.0 per cent.	133.3 per cent.	133.3 per cent.
1200.0	233.3	100.0
Averages: $\frac{1400.0}{}$	$\frac{183.3}{}$	$\frac{116.6}{}$

A similar preparation of agar and cystin gave the following as an average of three tests:

Agar 90—Cystin 10.

Water.	HCl $N/100$.	NaOH $N/100$.
2333.3 per cent.	583.1 per cent.	328.6 per cent.

A similar mixture of agar and urea (agar 90 parts, urea 10 parts) gave the following:

SWELLING.		
Water.	HCl N/100.	NaOH N/100.
2173.0 per cent.	716.6 per cent.	560.2 per cent.

Urea, the amino-acids, gelatine, albumen, and the saline soluble proteins of the bean dissolved with agar and dried into thin plates produced a greatly enhanced imbibition in water, an imbibition in hundredth normal hydrochloric acid not more than a third of that in water, while it was invariably less in alkaline than in acidified solutions. The interest in swelling which begins with a neutral desiccated section is however much less than that which attaches to the behavior of such material under changing conditions of alkalinity and acidity which are taken to occur in the living plant.

Dried plates of agar-protein, agar-tyrosin and agar-cystin .12 to .25 mm. in thickness and 3 by 4 or 5 mm. were placed in trios on the bottoms of stender dishes. Triangular pieces of glass were placed to cover the sections of colloid in each dish and an auxograph was arranged to give a bearing contact of the swinging arm on a socket in the center of the triangular plate. So long as the preparation remained in this condition the pen of the instrument traced a horizontal line on the sheet carried by the drum. Dried sections of the colloids have a very limited capacity for imbibition of acid and alkaline solutions, and hence it was desirable to start swelling or "growth" by an initial immersion of an hour in distilled water, which was poured in the dishes. After enlargement had begun hundredth-normal acid or alkaline solutions were used in alternation at intervals of one to three hours, as many as four changes being made in some cases before the total swelling capacity was reached. The results met all expectations based on theoretical considerations and the auxographic tracings might easily be mistaken for records of the variations of the length of a joint of *Opuntia*, for example.

Sections of plates 90 parts agar to "10" of tyrosin gave a tracing traversing 12 mm. vertically on the record paper during the first hour immersed in distilled water, remained stationary making a horizontal line during the second hour, the water having been

replaced with hundredth-normal hydrochloric acid, traversed 11 mm. of the scale in the third hour during which it was immersed in hundredth-normal sodium hydrate, then shrank 5 mm. in an hour in acid, then enlarged 9 mm. in three and a half hours in alkali, after which it shrank 3 mm. between 8:30 P.M. and 7 A.M. in acid. A change to alkali gave an enlargement of 6 mm. in two hours (Fig. 12). The auxograph was set to multiply so that the actual

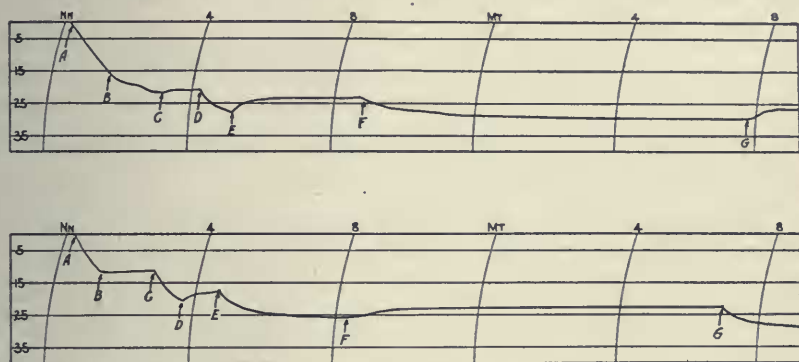


FIG. 12. Auxographic record of changes in section of agar 90—tyrosin 10, .14 mm. in thickness. Immersed in water at *A*, alkali at *B*, acid at *C*, alkali at *D*, acid at *E*, alkali at *F*, and acid at *G*. (Upper half of figure.) $\times 10$.

Auxographic record of changes in section of agar 90—tyrosin 10, .14 mm. in thickness. *A* in distilled water, *B* acid, *C* alkali, *D* acid, *E* alkali, *F* acid, and *G* alkali. (Lower half of figure.) $\times 10$.

enlargement in the periods noted was one twentieth of the distance traversed by the pen. The change from acidity to alkalinity is followed by the most marked effects when the colloid has taken up a fourth or a third of the possible total amount of water. Perhaps the most striking feature is the response of the colloid to acidification under the alternating conditions. Desiccated sections give a greater total swelling in acid than in alkali, but when a certain amount of swelling has already taken place under neutral or alkaline conditions no further increase in acid solutions and actual shrinkage ensues. A change to alkalinity is always followed by increased imbibition. Sections of plates containing 90 parts agar and 10 parts of gelatine gave results similar to those of the tyrosin mixture. No determinations of the minimum proportion of nitrog-

enous matter necessary to cause an agar mixture to behave in this manner were made. Ordinary agar contains some nitrogen and salts,¹⁶ and it is possible that the varying amounts might cause some disagreement of results obtained by the use of different lots of this substance.

The series of experimental trials with colloids which might display some of the fundamental physical properties of protoplasm of plants has resulted in finding that a mixture of substances of two of the three more important groups of constituents, carbohydrates and proteins, shows the imbibitional behavior of tissues and tracts of protoplasts of the plant. The differential action of such colloidal masses in distilled water, acid and alkaline solutions yields many striking parallels with growth. The changes from acidity to alkalinity have, so far as this type of experiment has been repeated, been made abruptly to avoid instrumental errors. Some acid or some alkali remained in the dishes when the change was made, and a certain amount of acid or alkali fixed or absorbed in the colloidal sec-

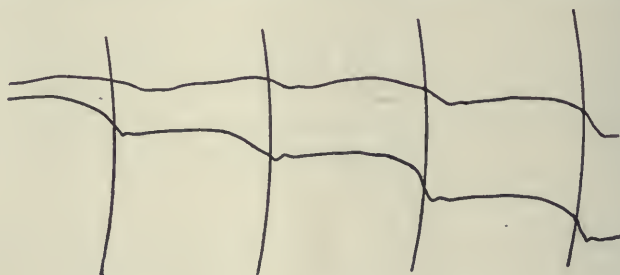


FIG. 13. Auxographic tracing of changes in length of shoot of *Opuntia* showing elongation and shortening (for comparison with Fig. 12).

tion, and neutralization, acidification or the reverse took place slowly with some formation of salts as might likewise occur in the plant (see Fig. 13).

It is through the relations indicated that metabolism or respiration may affect growth by the modification of imbibition capacity. Thus the accumulating surplus of acid in *Opuntia* begins to lessen by disintegration at daybreak and the decrease continues until about

¹⁶ See Noyes, H. A., "Agar for Bacteriological Use," *Science*, Vol. 44, No. 1144, p. 797, 196.

4 P.M. Whether complete neutralization or alkaline conditions ever occur naturally in this plant is doubtful.

The notable augmentation of imbibition which accompanies complete destruction of the balance of acid in the shoot of *Opuntia* under experimental conditions has already been described on p. 295. It has also been found that the mid-afternoon checking of growth characteristic of shoots of *Opuntia* which have accomplished a fourth or a third of their development, did not appear in the single bud, the development of which from a starved joint has been followed since the section of this paper dealing with growth was written.

The almost rhythmic undulations of the auxographic tracing of the elongation of a wheat leaf corroborated by measurements with the horizontal microscope suggest that growth in this organ may be accompanied by metabolic processes by which the balance of acidity and alkalinity falls now on this and then on that side, there being of course periods in which the growing protoplasts or some of them were in a neutralized state. During this time of course imbibition might be four to eight times as great as in either acid or alkaline conditions.

The change from any one of these conditions is of course accompanied by variations in imbibition. The character of the change is readily recognizable in the swelling of colloids, and it is believed that similar interpretations of the auxographic record of growing organs will be possible. The colloidal sections used for experimentation have a general identity with cell-masses except as to the lipid constituents. The part which these substances might play in the mechanics of growth can not as yet be made the subject of profitable conjecture. The analogies as to the action of the salts to be found in plants are also yet to be determined, and probably involve some of the phenomena studied as "antagonisms."

The striking similarities in behavior between the pseudo-protoplasmic material and cell-masses makes possible some new correlations in metabolism, imbibition and growth. It is hardly necessary to add in conclusion that whatever measure be given the contributions embodied in the present paper, the results presented do not

lead to any simplification of the major processes under discussion.

The advance is in a diametrically opposite direction. Newly determined features of carbohydrate metabolism included in respiration and necessary for growth and functioning have been found to be extremely complex. Imbibition in the plant is not that of a single colloid, and swelling is not the simple resultant of the action of two or more substances. The interaction between two emulsoids presents many possibilities. The proteins viewed physiologically appear to act as "sensitizers" to the carbohydrate gels which make up the greater part of the bulk of the protoplast, and to produce in them highly specialized effects with acids, alkalies and neutral solutions. The general character of respiration, and the nature and amount of its by-products acting upon a "sensitized" protoplasmic gel may be taken to determine the general aspect, rate, course and amount of growth in plants.